The Journal of Parasitology

Volume 33 APRIL, 1947 Number 2

THE EARLY DEVELOPMENT OF THE ROSTELLUM OF *CYSTICERCUS FASCIOLARIS* RUD., AND THE CHEMICAL NATURE OF ITS HOOKS

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INTRODUCTION

Clapham (1942) has made the interesting observation that the large hooks of *Multiceps otomys* Clapham develop from "two centres of chitinization," and the writer has shown (Crusz, 1944), by statistical analysis, that in the immature stages of *Multiceps serialis* (Gervais) there exists no correlation between the length of a hook and the ratio of its base to projected length. These observations, as well as many unsettled points and gaps in our knowledge of the development and chemical nature of taeniid hooks, have led to a consideration of some problems concerned with the early development of taeniids.

Detailed accounts of the development of rostellum and rostellar hooks were given by Leuckart (1856) for *Cysticercus pisiformis* Zeder, Goldschmidt (1900) for *Echinococcus* and Gläser (1909) for *C. longicollis* Rud. Young (1908) described the histodifferentiation of the hooks of *C. pisiformis*. Bartels (1902) described four stages in the development of *C. fasciolaris* Rud., but dealt neither with the early development of the hooks nor with some of the more important details of rostellar development. He stated: "Da mein ursprünglicher Plan, die Entwicklung des *Cysticercus fasciolaris* lückenlos vom Stadium der hohlen Blase bis zur fertigen Finne zu verfolgen, an den negativen Resultaten der von mir an Mäusen vorgenommenen Invasionsversuche scheiterte." Gläser (1909), reviewed all previous work, and concluded in regard to *C. fasciolaris:* "Wir können daher wohl annehmen, dasz auch der nicht beschriebene Teil der Entwicklung ähnlich wie beim *Cysticercus longicollis* verläuft."

The present work was started at the suggestion of Prof. D. R. R. Burt, Head of the Department of Zoology, University of Ceylon, to whom I am grateful for his kind advice and criticism. My thanks are also due to Dr. Phyllis Clapham of the Institute of Agricultural Parasitology, England, for helpful suggestions and references; to the Director of the Zoological Survey of India for having kindly loaned me much of the literature required; to Mr. L. D. Smith, Lecturer in Chemistry, University of Ceylon, for the carefully prepared photomicrographs; and to Messrs. A. S. Navaratnarajah and B. J. P. Alles, Assistant Lecturers in Chemistry, University of Ceylon, for their help in connection with some of the chemical aspects of this paper.

Received for publication, June 22, 1946.

MATERIAL AND METHODS

Infection of the liver with *Cysticercus fasciolaris* is a common feature of rats in Colombo. 2583 rats (*Rattus rattus kandianus*) taken from Colombo and its various suburbs, were examined, and 393, that is 15% of them, were found to be visibly infected with this parasite. Although mature strobilocerci were the most common, earlier stages were found in sufficient numbers to lend themselves to study by the usual methods.

Small pieces of liver, each with an encysted cysticercus, as well as scolices or scolex-rudiments dissected out from cysts, together with portions of the bladder-walls, were fixed in various fluids such as Bouin's, Carnoy's, corrosive sublimate, Gilson's, Zenker's and Helly's. The material was embedded in paraffin or doubly embedded in celloidin and paraffin. Sections were cut from 5 μ to 16 μ thick, and stained in Delafield's haematoxylin with or without eosin, Weigert's haematoxylin, Dobell's haematein, and Mallory's triple stain. For very early stages, especially for observing the structure and distribution of the rudimentary hooks, material fixed in Bouin and stained in Delafield's haematoxylin alone, gave as good results as that fixed in Zenker or Helly and stained in Mallory. In the latter case, however, sharp selective staining was obtained, the young hooklets and spines being stained a deep orange, in contrast to the cuticle which was stained blue. For later stages a combination of Zenker, Helly, or Gilson's mercuro-nitric fixative and Mallory's stain was unrivalled. Gilson's fixative softened the hooks to some extent so that it was possible to section even paraffin-embedded material. Stiles' N-butyl alcohol method of dehydrating after fixing in Gilson's fluid, was also tried in order to overcome the brittleness of the hooks, but this method did not appear to have any special advantage over the former. Double embedding in celloidin and paraffin, however, gave the best results.

DEVELOPMENT OF ROSTELLUM

The very early stages in the differentiation of the scolex-anlage do not differ essentially from those already described by Bartels (1902). The wall of the bladder is made up of an external cuticular layer within which lies a band of cells lining the cavity. These cells lie more or less irregularly, their nuclei being vesicular and their cytoplasm drawn out into processes of varying length. A distinct subcuticula is nowhere to be found. At a particular spot on the bladder-wall an active cell-proliferation has occurred in the youngest cysticercus studied. This portion of the wall appears thicker than the rest and bulges somewhat into the lumen. An area of most active cell-division in the center of this region, as shown by the consequent compactness of the cells (Bartels), was not observed. This is in agreement with Gläser's statement in regard to the description given by Bartels: "Ich glaube auch nicht, dass dort ein Wachstumsmittelpunkt liegt, sondern dass die Vermehrung der Zellen vom Blasenrand nach innen erfolgt." It would also seem that the scolex-anlage can be formed from any portion of the bladder-wall, but in cysticerci lying more superficially in the liver, it was noticed during dissection that this originated in most cases from the region of the bladder-wall not adjacent to liver-tissue.

The next stage of development consists in further increase in the number of scolex-cells, which now form a knob-like mass protruding into the cavity of the bladder. Hand in hand with this, there takes place an invagination of the cuticle close against the knob. This cuticular invagination goes on steadily and results in

the hollowing out of the once solid cell-mass. By this time the subcuticular layer of cells has become differentiated from the rest of the parenchyma. These cells elongate and lie perpendicular to the cuticle, especially in the region of the scolex.

At a later stage, which Bartels (after Hofmann) described as being 35 days old, the scolex is a bud-like outgrowth 0.27 mm by 0.17 mm. The cuticle lining its lumen is considerably thicker than that around the bladder-wall, and presents an uneven margin. The cuticle lining the floor of the bud is more or less horizontal at this stage. Cells are found in larger numbers at the floor of the invagination and these gradually decrease in number towards the neck. The subcuticula is now easily recognizable. A fibrous receptacle investing the whole scolex-rudiment is not formed during these early stages of development. This is in agreement with the earlier accounts of Raum (1883) and Bartels (1902). Moreover, from an examination of a very large amount of material, both in the fresh condition as well as after fixation, it seems that the scolex-plug is constructed obliquely in respect of the bladder-wall (Raum, 1883) and not perpendicularly (Bartels, 1902). The degree of obliqueness differs, however. The typical flexure occurring early in the development of *C. longicollis* (Gläser, 1909) takes place in *C. fasciolaris* only after the scolex has been almost completely differentiated. Bartels (1902) discontinued his description at the stage just described and resumed it long after the development of hooks and the rostellar rearrangements had taken place.

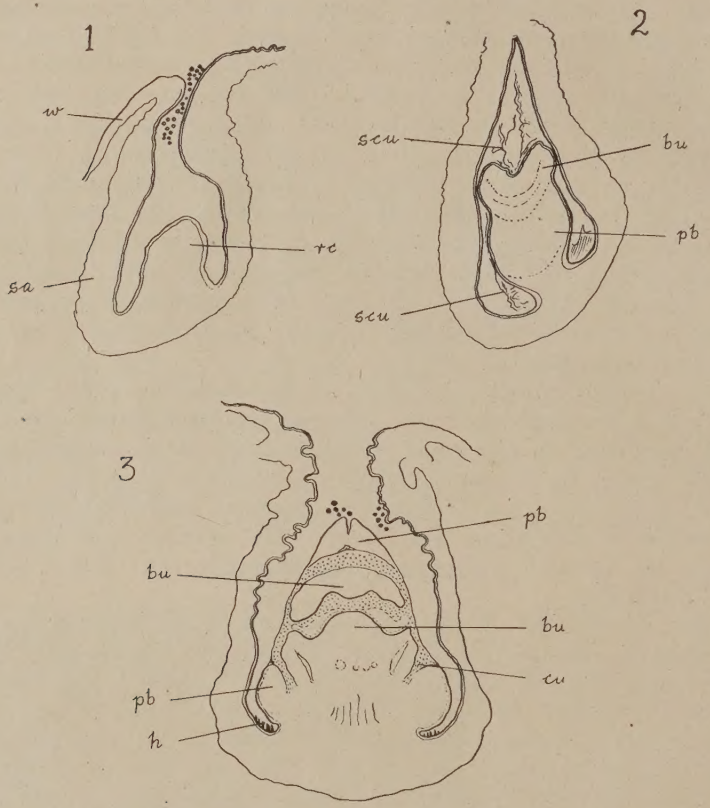
As development proceeds there occurs a further elongation of the hollow bud and an increase in its cellular make-up. The most important feature, however, is the arching upwards of the cuticle and cells of its floor. This rostellar elevation develops on the same lines as that described by Gläser for *C. longicollis*. The arrangement of the cells takes on a characteristic appearance, setting themselves always in the direction of proliferation and growth. The elevation is at first somewhat hemispherical, but later on it becomes conical (Fig. 1, rc). This is the rostellar cone (Rostellarkegel).

In his account of the ontogeny of *C. longicollis,* Gläser described and figured a stage when this rostellar cone becomes a double cone (Doppelkegel), that is, the whole rostellar eminence assumes the shape of a diamond in long section, being broadest in the middle. A slightly later stage was observed for *C. fasciolaris*. The sinking of the upper cone or bulb has already begun, so that it has already lost its conical shape if it had any (Fig. 2, bu). The lower cone or prebulbar region (Präbulbares Scheitelfeld) preserves its shape to some extent. A still later stage shows the cuticle growing in and making its way between the bulb and prebulbar region, the bulb having now sunk deeper into the underlying mass of cells (Fig. 3). These cells take on a somewhat different aspect from the other cells that belong to bulb and prebulbar region. One characteristic of this group of cells is the paucity in number and the consequent dearth of deeply staining nuclei.

Fig. 10 shows the bulb as a distinctly globular structure surrounded by the prebulbar region. The cuticle separating the two is clearly visible, especially in stained preparations where it is stained blue by Mallory. By now the cavity of the entire scolex-anlage has been considerably enlarged, whereas in earlier stages the rostellar cone and the later formed double-cone often occupy the greater portion of the lumen and even touch the wall of the bud.

Fig. 11 shows a further stage in rostellar development. The bulb has now sunk

completely; its edge is turned in owing to the pressure exerted by the surrounding tissues especially those of the prebulbar region, which has now come to lie closely over it. Later on, the prebulbar region, carrying the hooks on its outside, coalesces over the bulb, in which appear the various muscular elements described by Bartels. This coalescence of the prebulbar region is mainly a fusion of its cuticle which has hypertrophied, and which appears here as a peculiarly vacuolated structure which led Gläser to regard it as liquefied cuticle. When the bulb begins to stretch itself



FIGS. 1 & 2. Longitudinal sections of early developmental stages of *Cysticercus fasciolaris*. (×72½.)

FIG. 3. Oblique longitudinal section of a later stage, showing sinking of bulb. (×72½.) *bu*, bulb; *cu*, cuticle; *h*, hooklet; *pb*, prebulbar region; *rc*, rostellar cone; *sa*, scolex-anlage; *scu*, moulted cuticle in lumen; *w*, bladder-wall.

and no longer lies curved as in Fig. 11, its cuticle also fuses with that of the prebulbar region above it, and presents the same structure. Below the bulb are developed tiers of muscles that form the muscular "cushion." The main structural details of a scolex of this stage of development, as well as of later stages, have been worked out by previous workers, especially Bartels (1902).

CUTICLE

The cuticle, which is a secretion of underlying parenchymatous cells, is present as the outermost layer of the bladder, long before the differentiation of the scolex-

anlage takes place. In material stained with Mallory it takes on a bright blue color and is of uniform thickness. It does not appear to be structurally complex at this stage. Where cell-proliferation takes place, however, it thickens considerably, and the normal invaginating cuticle which is always thicker than the rest, presents an undulate margin and is covered by a coat of fine hair-like processes. In later stages,

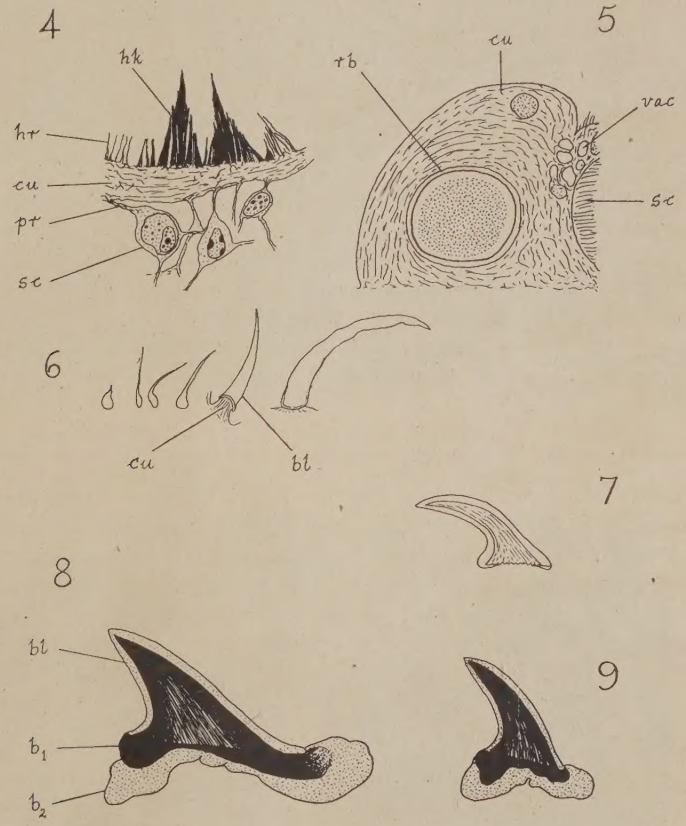


FIG. 4. Longitudinal section through portion of cuticle and subcuticula of *C. fasciolaris,* at the time of appearance of hooklets. (Leitz ocular 20, obj. 1/12.)

FIG. 5. Longitudinal section through portion of hypertrophied cuticle around prebulbar region of *C. fasciolaris,* at the time of thickening of hook-walls. (Leitz ocular 10, obj. 1/12.)

FIG. 6. Stages in the development of hooks. (×400.)

FIG. 7. Small hook, before development of base. (×90.)

FIGS. 8 & 9. Large and small hooks of a fully developed *C. fasciolaris,* after treatment with sodium plumbite, to show presence of sulphur-bearing radical in the hook-substance. Note also that the hooks are considerably swollen. (×90.) b_1, proximal region of base; b_2, distal region of base; *bl,* blade; *cu,* cuticle; *hk,* hooklet; *hr,* cuticular "hairs"; *pr,* protoplasmic process of subcuticular cell; *rb,* round vacuolar body; *sc,* subcuticula; *vac,* vacuole.

and under an oil immersion lens, the cuticle is seen to be formed of a mesh-work of fine fibers which are very often continuous with the protoplasmic processes of underlying subcuticular and parenchymatous cells (Young, 1908). In the interstices of this mesh-work is the homogeneous cuticular substance which has probably been secreted by these fibers and processes. The cuticular hairs, wherever distinctly visible, are seen to be similar processes which extend beyond the cuticle (Fig. 4).

The cuticle thickens most on the bulb, prebulbar region and on the stretch of tissue lying between these and the suckers. This intermediate region becomes considerably enlarged and forms a mound-like eminence which projects into the cavity, around the prebulbar region, and ultimately outgrows the latter in height as well as in bulk. Between this eminence and the prebulbar region there is now a deep fold which comes to be occupied by the definitive hooks which are growing larger. Gläser mentioned an unusual hypertrophy of the cuticle and also a thickening and elongation of the subcuticula throughout the entire extent of this elevation, during the growth of the definitive hooks in *C. longicollis.* In *C. fasciolaris,* on the other hand, the cuticle and subcuticula of this region do not assume proportions that differ markedly from those of the bulb and prebulbar region. Only after the hooks have developed their bases, does this cuticular enlargement occur (Fig. 5).

At the time of hook-formation the cuticle in the regions beyond the eminence and beyond the suckers, becomes consolidated and assumes a more or less permanent structure: below the layer of hairs is a very narrow pellucid zone, followed by a broad granulated layer, the innermost region of which is provided with clear oval vacuoles (Rössler, 1902; Young, 1908). As has been remarked by Leuckart (1886) and Young (1908) for *C. pisiformis,* the cuticle of the developing scolex of *C. fasciolaris* appears to scale off on the surface, and portions of shed cuticle are to be found in its lumen (Figs. 2 & 11).

HOOKS

Long before the appearance of the definitive hooks, the cuticle lining the rostellar cone and the later formed double-cone, and the cuticle around the base of the rostellar elevation, are covered with minute points or hooklets, which stain intensely with haematoxylin as well as with Mallory. These hooklets are formed among the cuticular processes and, in very early stages, each of them is seen to be formed by a cementing together of groups of close-set cuticular processes or "hairs" (Fig. 4, hk) in the manner described by Young (1908) for the hooklets of *C. pisiformis.* Soon, however, as more cuticular substance is secreted they stain more intensely and appear as single discrete units.

The distribution of these hooklets is not at all regular in the early stages, and sometimes they may not be developed until after the rostellar rearrangements have been initiated. At the time of the sinking of the bulb they are easily recognized, and their distribution is seen to be limited. They are found in large numbers on the entire prebulbar region (Figs. 10–12), and over the whole extent of the cuticle around the prebulbar region, up to but not including the suckers. Some hooklets are to be found even on the bulb, but they disappear very quickly, though a few may survive even up to the period of coalescence of bulb and prebulbar region (Fig. 11). The distribution of hooklets seems, therefore, to be different from that described for *C. longicollis,* where they are not found as far as the suckers, but already stop short at the top of the eminence between suckers and prebulbar region. As regards the occurrence of hooklets on the bulb, there seems to be no reason to doubt that precisely the same condition is to be found in both *C. longicollis* and *C. fasciolaris.*

The definitive hooks are formed from hooklets lying at the bottom of the fold around the prebulbar region (Figs. 10 & 11). These hooklets increase both in length and girth as more and more hook-substance is secreted. From the fact that increase

FIG. 10. Photomicrograph of a portion of longitudinal section of *C. fasciolaris*, showing bulb, prebulbar region (with minute hooklets); and two young definitive hooklets, each developing around a conical extension of the cuticle. (Zenker: Mallory.) (×500.)

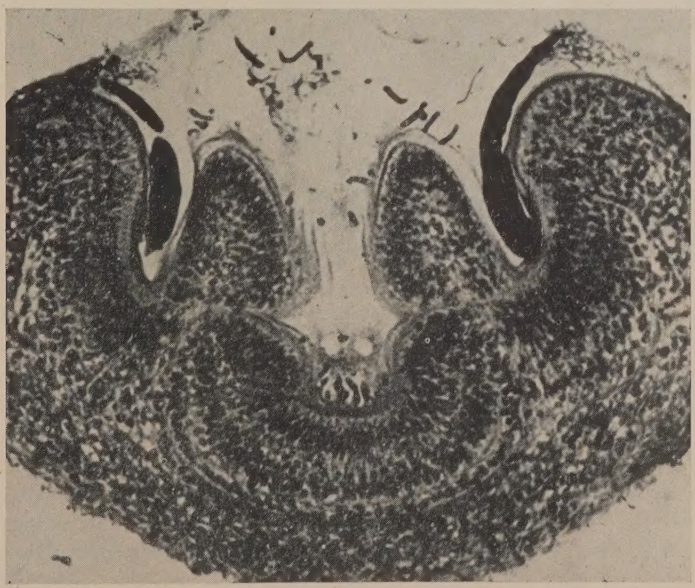


FIG. 11. Photomicrograph of a portion of longitudinal section of *C. fasciolaris*, at a more advanced stage, showing prebulbar region closing over sunken bulb. Note the large, thin-walled definitive hooks, the numerous rudimentary hooklets of prebulbar region and region beyond it, and the few hooklets on the bulb itself. (Bouin: Delafield's haematoxylin.) (×300.)

in girth takes place from the base towards the apex (Fig. 6), it would appear that this secretion is an internal process occurring from within the hooklet (Leuckart). This is in keeping with the observations made on the mode of origin of the hooklets themselves, namely, by the cementing together of cuticular processes and continued secretion of cuticular substance. Another feature that supports this is the fact that each developing hooklet of a slightly later stage is a hollow claw-like structure formed around a conical extension of the cuticle (Fig. 6, cu). This is especially evident in sections stained with Mallory (Fig. 10), where the cuticle as well as the conical extensions into the hooklets are stained blue, and are sharply differentiated from the hooklets themselves which are stained orange. This conforms to Leuckart's description (1856): "Sie erscheinen als hohle dünnhäutige Kegel, die in ähnlicher Weise wie die Hörner der Antilopen auf dem Knochenfortsätze des Stirnbeins, so auf einer könischen Verlängerung der Epidermis aufsitzen."

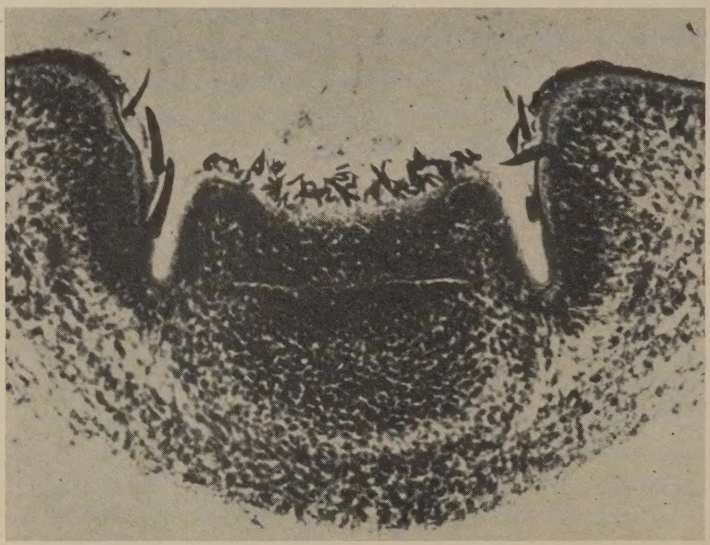


FIG. 12. Photomicrograph of a portion of longitudinal section of *C. fasciolaris*, at a slightly earlier stage than in Fig. 11, and not passing medially. Note prebulbar region and region beyond it, covered with hooklets. (Bouin: Delafield's haematoxylin.) (×300.)

The hollow claw-like definitive hooks gradually grow out into large thin-walled hooks. The walls then begin to thicken after the blade has attained a certain size. Gläser maintained that this thickening is due to the deposition of more hook-substance on the outside of the hook, and he inferred this from the highly vacuolated and intensely staining nature of the cuticle, and the presence of intensely staining "eigentümliche runder Körper," in just those regions where the thickening blade is in contact with the hypertrophied cuticle. He attributed to these peculiar bodies a function connected with the secretion of hook-substance, for they are said to be most prominent at the time of the thickening of the walls of the hooks, and to disappear when the hooks are apparently fully formed.

Against the theory of the internal deposition of hook-substance, maintained by Leuckart (1856), Gläser brought forward the above facts, as well as his observation

that there exists a sharp distinction between the base of the claw-like blade and the cuticle, there being no conical cuticular extension into the hollow of the hook.

Nothing can be adduced against Gläser's observations in respect to the highly vacuolated nature of the cuticle and the presence of the round granular bodies, until the exact nature and function of these structures are determined by histochemical methods. The round bodies were observed in many sections of more advanced scolices and appear to be large vacuolar structures filled with a fine granular substance, with a clear space around each of them (Fig. 5, rb). They do not stain intensely with haematoxylin or Mallory.

Although the mode of thickening of the wall of the blade may bear out further investigation, the preliminary growth of the minute hooklet into the thin-walled definitive hook appears to be an internal process, the main principle of hook-formation being the cuticular extension into the hooklet, the presence of which extension, though denied by Gläser, is proved beyond doubt by proper selective staining (Fig. 10).

The origin and development of the handle and guard (base or roots) of a hook take place at the base of the thickened blade. A fresh deposition of hook-substance takes place around the base and this gives rise to a "basal plate," or more correctly "basal ring," which develops extensions in opposite directions forming the guard and handle. This is a result of unequal rates of deposition, the secretion being more vigorous in those regions of the basal ring which grow out into the roots. The basal ring as well as the roots are embedded in the cuticle and almost from the very beginning appear as thickened structures staining very intensely with Mallory. No "round bodies" are to be seen in the cuticle of this region, but the cuticle appears to be vacuolated. Further, there is not the slightest indication in any of the sections, of the presence of "two centres of chitinization" for the large hooks, as observed by Clapham (1942) for *Multiceps otomys*.

CHEMICAL NATURE OF HOOKS

Little or nothing is known about the chemical nature of cestode hooks. Most authors refer to them as being chitinous, although there appears to be no experimental evidence whatsoever for such an assumption. Dollfus (1942) has carried out a few tests on the hooks of the proboscides of tetrarhynchids. He stated: "Il est facile de reconnaître que les crochets des trompes ne se comportent pas vis-à-vis des réactifs et colorants histologiques comme de la chitine; leur substance ne paraît pas apparentée à ce glucide . . ." and came to no definite conclusions about the nature of the hook-substance.

Preliminary tests were therefore carried out in the course of this work, with a view to ascertaining the chemical nature of the hooks of *C. fasciolaris*. Campbell's modified van Wisselingh-Brunswik tests for chitin (Campbell, 1929), which have been used more recently by von Brand (1940), Dennell (1943), Thomas (1944) and others, and which are believed to be by far "the most dependable microchemical tests for chitin," were the first to be used in this investigation. Living strobilocerci were decapitated, and pieces of scolices with well-developed hooks were subjected to the usual treatment for about 20 minutes with KOH solution heated to 160° C in a glycerine bath. As controls, pieces of the exoskeleton of the prawn *Penaeus*, as well as portions of the colony of *Bugula flabellata* were also subjected to the same

treatment. The results were significant at the very outset. Whereas the pieces of crustacean exoskeleton and certain parts of the bryozoan material were entirely resistant to the superheating with KOH, and responded in the typical way to the subsequent acetic acid–sulphuric acid test, and yielded the violet color and the typical spherites of chitosan sulphate(?) in the microchemical test with iodine and sulphuric acid, the entire scolices, together with the hooks, of *C. fasciolaris* disintegrated and dissolved in the KOH solution. This immediately proves the absence of chitin in the hooks of *C. fasciolaris*. Chitin was undoubtedly present in the crustacean and bryozoan material, as was shown by the spherites obtained, of which permanent preparations stained in acid fuchsin, were made.

Some of the well-known tests for proteins and their derivatives were next tried out for any specific color reactions. Dollfus (1942) tried out a few of them on preserved tetrarhynchid material, but obtained very meager results owing to the minute size of the hooks and the difficulties of observation. In the present investi-

TABLE 1

Reaction	Result	Conclusion
Biuret	b_2 of base (see Figs. 8 & 9) assumes a distinct purple coloration. The most superficial layer of blade also assumes a purplish tinge.	Indicates presence of *protein* or a long polypeptide chain.
Xanthoproteic	Entire hook turns yellow in cold conc. HNO_3 and the color deepens with heating. Addition of a drop of alkali (ammonia) turns it to orange.	Generally indicates presence of *tyrosine*, *phenylalanine* or *tryptophane*, and is characteristic of the phenyl radical present in these acids.
Millon's	b_2 of base alone turns bright red in cold Millon's reagent. It turns to a deep red color with heating, the most superficial layer of the blade also assuming a light pinkish tinge.	The red coloration is specific for the mono-hydroxy benzene group, but is generally considered specific for *tyrosine*.
Sakaguchi's	b_2 of base as well as the most superficial layer of blade turn distinctly red.	Indicates presence of *arginine*.
Liebermann's	No color developed.	Indicates absence of tryptophane.
Sodium plumbite	b_1 of base and inner layers of blade turn pitch black. b_2 of base and outermost layer of blade turn dark brown.	Indicates presence of relatively high percentage of loosely combined sulphur, invariably due to the presence of the sulphur-bearing radical *cystine*, in which the keratins are especially rich.

gation the experiments were carried out on fresh material. The living strobilocerci were decapitated, and the scolices were washed thoroughly in distilled water. They were allowed to remain for some time in fresh distilled water, after which the hooks were easily detached, collected and washed repeatedly in several changes of distilled water. The color reactions were observed both under the binocular microscope and under the low power of the compound microscope. Blank tests for purposes of comparison were carried out on pieces of ox horn, human nail, gelatin and egg albumin. Table 1 summarizes the results obtained. These results do no more than indicate that the hook-substance closely resembles a scleroprotein of the keratin type. This is further supported by the fact that it is a product of "epidermal activity"; that it resists many chemical reagents and is insoluble in water, dilute acids and alkalies; that it is not attacked by pepsin, trypsin or bacterial proteinase (as is obvious from the effects of the digestive enzymes of the hosts on the hooks of both living and dead cestodes, and the resistance of the hooks to decomposition); and, finally, that it is attacked with remarkable rapidity by the sulphides of the alkalies and alkaline earths. These are all characteristic properties of the keratins. This preliminary investigation will have, however, to be followed up by detailed qualitative and quantitative

analyses of a large amount of material before one could arrive at anything more definite as regards the actual chemical structure of the hook-substance.

CONCLUSIONS

Very little doubt can attach to the identity of the species dealt with in this study. The fact that cysticerci infest the liver of the rat, that early scolex-rudiments possess no fibrous "receptaculum," that strobilocerci of various ages are often to be found in conjunction with earlier stages where strobilation has not yet begun, and that the shape and size of the hooks are typically those of *C. fasciolaris,* clearly indicates the species.

The scolex-rudiment of *C. fasciolaris* grows out obliquely in respect of the bladder-wall and lacks a "receptaculum" at earlier stages of development. The internal changes involved in the development of the rostellum proceed on the same lines as those described by Gläser for *C. longicollis,* except for the distribution of the rudimentary spines or hooklets which occur in very large numbers on the prebulbar region as well as on the entire cuticle around this region, even as far as the suckers. The suckers however do not possess hooklets. The bulb undoubtedly bears hooklets which disappear very quickly, as probably is the case in *C. longicollis,* though Gläser was doubtful of it. Gläser observed that the distribution of hooklets does not proceed beyond the top of the eminence surrounding the prebulbar region in *C. longicollis,* and so disagreed with Leuckart (1856) who mentioned their presence throughout, even on the cuticle of the suckers, in *C. pisiformis.* It is difficult to state at present whether the differences so far seen in the distribution of hooklets in these three species, are specific differences. The case of *Echinococcus* seems to present a real difference. The entire bulb, like the other regions, is covered with numerous hooklets for a long time during early development. The rapidity with which rostellar development takes place in cysticerci appears to be correlated with the paucity of hooklets on the bulb and their early disappearance, as Gläser has correctly viewed it.

Regarding the origin and development of the hooklets as well as of the definitive hooks, it seems clear that Young's account of the origin of hooklets by a cementing together of cuticular processes is in agreement with the observations made in this study. Further growth of the hooklet into the definitive hook is by further secretion of hook-substance from this initial conical structure. Leuckart's conical extension of the cuticle into the hollow of the hook is a very real feature as is borne out by the present work. The mode of thickening of the hook-wall and the nature of the vacuoles and round granular bodies of the cuticle have yet to be investigated. The roots of the hooks grow out as a result of fresh deposition of hook-substance around the base of the blade, the deposition being more vigorous in just those regions where the roots normally arise. A "second centre of chitinization" for the handle of the large hooks was not observed for *C. fasciolaris.* This is probably so even for other cysticerci.

By application of Campbell's modified van Wisselingh-Brunswik tests for chitin, the hook-substance of *C. fasciolaris* is shown to be not of the nature of chitin. Other tests seem to indicate that it is a scleroprotein of the keratin type.

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STUDIES ON THE GENUS *HYMENOLEPIS*, WITH DESCRIPTIONS OF THREE NEW SPECIES

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A collection of cestodes from Texas shore birds, obtained from 1941 to 1946, yielded three new species of the genus *Hymenolepis*. In addition to descriptions of the new species, two questions are discussed: the synonymy of *Taenia fusus* Krabbe, and the recognition of subgenera within *Hymenolepis*. My thanks are due to Dr. Asa C. Chandler, who directed the study, and to Dr. R. Chester Hughes for his expression of opinion on the synonymy.

Hymenolepis (*Hymenolepis*) *hughesi* new species

Diagnosis: Characters of the subgenus. Strobilae containing gravid proglottids 37 to 50 mm long, with a maximum width of 1 mm and with 120 to 150 proglottids. Longitudinal muscles in two distinct layers, the inner consisting of ten bundles, of which the dorsomedian pair is large and the other eight moderate in size, the outer layer consisting of about thirty-two small bundles. Scolex 144 to 173 μ wide; rostellum 40 to 54 μ wide bearing ten hooks 14.2 to 15 μ long; unarmed suckers 64 to 82 μ in longitudinal diameter. Genital pores chiefly unilateral on right side.

Fusiform cirrus pouch 158 to 180 μ long by 45 to 53 μ in diameter in mature proglottid; cirrus short, unspined, with expanded tip when extruded, and with slender stylet 173 to 219 μ long. External seminal vesicle small, immediately aporal to cirrus pouch; vasa efferentia joining testes at anterior margins; testes irregularly placed, but always in more or less of a transversal row, median testis sometimes aporal to vitelline gland, sometimes poral to vitelline gland, sometimes median; testes attaining 124 to 164 μ in diameter. A single small accessory sac posterior to genital atrium.

Vagina ventral to cirrus, passing laterally as a slender duct to large seminal receptacle; seminal receptacle anterior to poral lobe of ovary, persistent in gravid segments. Bilobate or trilobate ovary attaining 278 to 400 μ in lateral extent, placed anterior to testes and median of proglottid; spherical or ellipsoid shell gland postero-dorsal to ovary; ellipsoid vitelline gland at center of proglottid, attaining 78 to 102 μ in lateral extent. Uterus arising as a narrow sac extending laterally from a point dorsal to center of ovary, finally filling entire segment; ellipsoid eggs 47 to 50 μ long; ellipsoid onchospheres 31 to 33 μ by 26 to 28 μ.

Host: Piping Plover, *Charadrius melodus*.
Location: Intestine.
Locality: Galveston Island, Texas.
Type: U. S. Nat. Mus. Helm. Coll. No. 45744.

Occurrence.—This description is based upon six specimens taken from two hosts. All but one fragment, which was sectioned, were mounted whole in balsam and stained with carmalum; measurements were made on all specimens.

Discussion.—The cirrus, cirrus stylet, and cirrus pouch develop early and are difficult to find shortly after the uterus begins to form. An unusual feature is the presence of four "offside" (i.e., on the left side) genital pores in one specimen and a single "offside" pore in another worm; this is not very uncommon in "unilateral" forms of Davaineidae, but violates a family characteristic of the Hymenolepididae. In each specimen one or two proglottids contain only two testes; in two worms a single segment contains four testes.

Closely related species may be differentiated from *Hymenolepis hughesi* as follows (data from original description, except as noted otherwise):

H. amphitricha (Rudolphi, 1819) Fuhrmann, 1906 (data from Clerc, 1903),

Received for publication, January 20, 1947.

H. annandalei Southwell, 1922, and *H. neoarctica* Davies, 1938 possess similar hooks, but have the testes arranged in a triangle and lack the cirrus stylet.

H. clavicirrus Yamaguti, 1940, *H. recurvirostrae* (Krabbe, 1869) Fuhrmann, 1906 (data from Huges, 1941) and *H. recurvirostrae magnosacco* Joyeux, Baer, and Martin, 1936 possess similar hooks and testes in a transversal row, but have a cirrus pouch that extends across the midline of the segment and lack the cirrus stylet. The latter two forms have a spined cirrus.

H. kowalewski Bacsynska, 1914 (data from Hughes, 1941) possesses hooks of similar shape and testes in a transversal row, but has smaller hooks and larger cirrus pouch, lacks a cirrus stylet, and has a much wider strobila and an ovary placed on the poral side.

H. recurvirostroides Meggitt 1927 possesses similar hooks and testes in a transversal row, but has a smaller, more persistent cirrus pouch and lacks a cirrus stylet.

H. fusa Krabbe (1869) Fuhrmann 1906 (data from Southwell, 1922 and 1930) possesses similar hooks and testes in a line, but a larger cirrus pouch and a smaller, more posteriorly placed ovary.

Of the fourteen species listed by Brock (1942) as possessing a cirrus stylet, not one has the combination of testes in a transversal row and hooks shaped as in the present species. *Hymenolepis hughesi* is the first species with a cirrus stylet reported from a Charadriiform host. However, inasmuch as the stylet is difficult to see, some of the poorly described forms may possess it.

This species is named in honor of Dr. R. Chester Hughes, whose recent work (1940, 1941) on this genus has proved very helpful.

THE IDENTITY OF *Taenia fusus* KRABBE 1869

In 1869 Krabbe described *Taenia fusus* from *Larus hyperboreus* and *Larus ridibundus*. But his description gave little modern taxonomic information save hook number, shape, and length. On the basis of these characters, Fuhrmann (1906 and 1908) assigned the species to *Hymenolepis*, without studying specimens.

Southwell (1922: 371) briefly noted five specimens from *Larus brunneicephalus* and at least six specimens from *Hydroprogne tchegrava* as *Hymenolepis fusus* (Krabbe, 1869) Fuhrmann, 1906, mentioning in his description only hook length and the presence of occasional segments with only two testes. It may be argued that this does not constitute an "indication" and, therefore, not a first revision. On the contrary, Southwell cited Ransom (1909) as authority for the subfamily HYMENOLEPIDINAE and, it may be assumed, studied Ransom's definitions of the subgenus *Hymenolepis* and genus *Hymenolepis* (Southwell, *op. cit.* page 377, recognized the subgenus *Echinocotyle* Blanchard); the latter was identical with that used by Hughes as recently as 1941. In other words, Southwell, a competent helminthologist, in 1922 studied a series of specimens which he differentiated from other species having internal and external seminal vesicles, three testes, unilateral genital pores, no spines on suckers, etc., by their having occasional segments with only two testes and ten hooks of the size and shape figured by Krabbe for *Taenia fusus*, to which species he assigned them. In the writer's opinion this constituted an "indication" and a first revision.

Linton (1927) assigned specimens from *Larus argentatus* to *H. fusus*. However, Davies (1938) showed that Linton's material, in common with his own from *Larus*

argentatus and *Larus marinus*, was not conspecific with Krabbe's because the hooks were too long. Rather, it belonged to a new species, *H. neoarctica* Davies 1938. Joyeux and Baer (1928) described specimens with a single testis, from *Larus argentatus*, as **Haploparaksis fusus* (Krabbe, 1869) Joyeux and Baer, 1928. Similar nomenclature was used by Fuhrmann (1932), who even assigned Southwell's records (Fuhrmann, 1932: 245–246) to **Haploparaxis*, and also by Davies (1938).

Hughes (1940) proposed a new name for Southwell's specimens, *Hymenolepis neosouthwelli*, which were obviously generically distinct from those of Joyeux and Baer, because redescribed and figured with three testes in 1930 (Southwell, 1930).

On the basis of the first reviser principle, the name and application used by Southwell (1922) must stand, and the cestodes described by Joyeux and Baer (1928) are without a name—**Aploparaksis* sp.

Krabbe's species is:

Hymenolepis fusa (Krabbe, 1869) Fuhrmann, 1906

Synonyms: Taenia f. Krabbe (1869); *Hymenolepis f.* Fuhrmann (1906 and 1908), Ransom (1909), Southwell (1922 and 1930); *H. neosouthwelli* Hughes (1940). *Not Hymenoplepis f.* Linton (1927) nor **Haploparaksis f.* Joyeux and Baer (1928).

Hymenolepis (*Echinocotyle*) *litoralis* new species

Diagnosis: Characters of the subgenus. Rostellum 56 to 71 μ wide, bearing ten hooks 86 to 87 μ long, the blade of each hook forming less than half its total length; suckers 91 to 106 μ

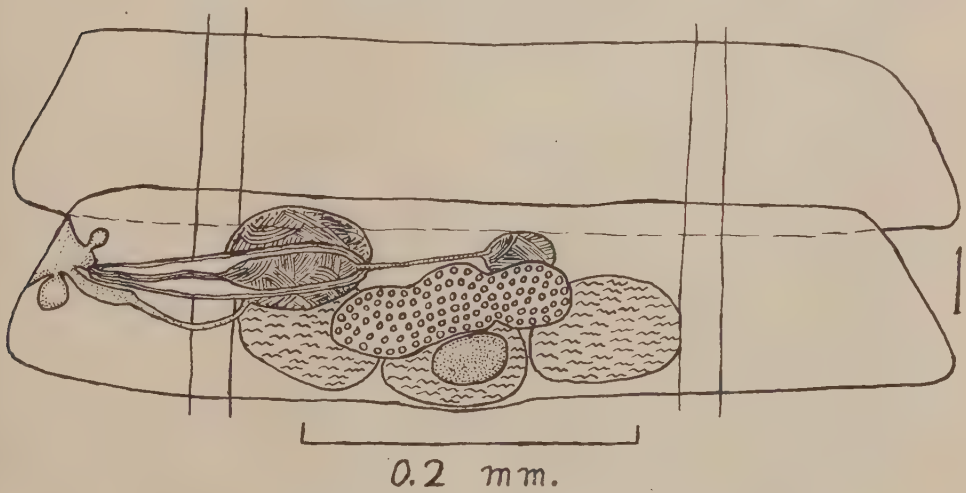

FIG. 1. *Hymenolepis litoralis* n. sp. Ventral view of mature segment.

in longtudinal diameter, sparsely armed with minute spines around edges and across center; scolex 175 to 237 μ wide. A long, slender neck present; strobilae with gravid proglottids 45 to 65 mm long, with maximum width of 0.9 mm. About thirty large bundles in inner layer of longitudinal muscles.

* Clerc (1903) erected the genus *Aploparaksis;* his barbaric spelling was corrected, in violation of Article 19 of the International Code of Zoological Nomenclature, by several later authors. Spellings here given are as in the paper cited, in each case.

Testes in a shallow triangle, lateral ones anterior, median one posterior (more pronounced in immature proglottids); testes symmetrical with respect to the median line of segment, about equal in size, attaining a diameter of 86 to 96 μ. Spherical external seminal vesicle antero-dorsal to ovary; cirrus pouch 153 to 208 μ in length by 33 to 36 μ in diameter in mature proglottid, extending across less than one-third of segment, with large internal seminal vesicle; cirrus when everted 129 to 135 μ long, armed with very small, closely set spines; two accessory sacs present, surrounded by only a few small glandular cells.

Vagina opening to genital atrium ventral to cirrus, but extending dorso-medially to large seminal receptacle dorsal to aporal part of cirrus pouch; ovary trilobate, attaining 146 to 218 μ in lateral extent; ellipsoid vitelline gland posterior to middle of ovary; lobate shell gland visible in partially gravid segments, after the testes and ovary have largely disintegrated, immediately aporal to seminal receptacle; uterus arising dorsal to ovary as a bilobate sac, finally filling about two-thirds of proglottid. Mature ellipsoid onchospheres 28 to 30 μ by 22 to 24 μ with three membranous coats; eggs 30 to 37 μ long.

Host: Sanderling, *Crocethia alba*.
Location: Intestine.
Locality: Galveston Island, Texas.
Type: U. S. Nat. Mus. Helm. Coll. No 45745.

Occurrence.—This description is based on seventeen specimens taken from five of eight Sanderlings. All were mounted whole in balsam and stained with carmalum. Complete measurements were made of the three specimens having scolices, and partial measurements of ten others.

Discussion.—*Hymenolepis litoralis* resembles most closely *H. nitida* (Krabbe, 1869) Fuhrmann, 1906. From that species as from all other species in the subgenus *Echinocotyle* except *H. uralensis* (Clerc, 1902) Fuhrmann, 1906, it may be distinguished by the position of the testes in an inverted triangle, rather than in a straight line or upright triangle. *H. nitida* also differs from the present species in possessing one accessory sac instead of two, and testes unequal in size instead of equal. *Hymenolepis* (*E.*) *uralensis* differs from the present species in possessing one accessory sac instead of two, and in having smaller hooks. The other species of the subgenus differ from these three in possessing much smaller hooks. (Data from Clerc, 1902 and 1903, and Hughes, 1941.)

Hymenolepis (*Echinoctyle*) *crocethiae* new species

Diagnosis: Characters of the subgenus. Strobilae 1 to 2.5 mm long, with sixty to eighty segments when gravid segments present. Inner layer of longitudinal muscles consisting of eight large bundles. Rostellum 40 to 51 μ in diameter, bearing ten hooks 86 to 98 μ long; scolex 182 to 219 μ wide; suckers 74 to 100 μ in longitudinal diameter, sparsely armed with minute spines around the edges and across the center; a short neck present.

Testes in a triangle with the aporal one anterior, and graded in size; poral testis attaining 21 to 27 μ; median testis 18 to 25 μ; aporal testis 11 to 21 μ. Spherical external seminal vesicle dorsal and aporal to cirrus pouch; internal seminal vesicle long and slender; cirrus pouch slender, cylindrical, 71 to 84 μ long by 17 to 18 μ in diameter in mature proglottid; cirrus spined, 48 to 49 μ long when extruded; two accessory sacs present, respectively postero-ventral and antero-dorsal to genital atrium, each surrounded by heavily-staining glandular cells.

Vagina opens ventral to cirrus, passes mediad, then posteriad to enter seminal receptacle; ovoid seminal receptacle poral to ovary, persistent in gravid segments; lobate shell gland dorsal to ovary; bean-shaped ovary aporal to cirrus pouch, attaining 39 to 52 μ in lateral extent; ellipsoid vitelline gland postero-ventral to middle of ovary; uterus arising dorsal to ovary as simple sac, finally filling about half of gravid segment. Eggs 19 to 22 μ long, with protruding lips at one end, and containing ellipsoid onchospheres 17 to 19 μ by 13 to 15 μ.

Hosts: Sanderling, *Crocethia alba* (type host), and Ruddy Turnstone, *Arenaria interpres*.
Locality: Intestine.
Locality: Galveston Island, Texas.
Type: U. S. Nat. Mus. Helm. Coll. No. 45746.

Occurrence.—This description is based on about 180 specimens stained with haematoxylin or carmalum and mounted whole in balsam. Measurements were

made on thirteen specimens from six host individuals. The species was taken from all of eight Sanderlings and a single Ruddy Turnstone.

Discussion.—Hymenolepis crocethiae differs from *H. nitida* (Krabbe, 1869) Fuhrmann, 1906 and *H. litoralis* Webster (data from Clerc, 1902 and 1903 and above) in possessing hooks of somewhat different shape. From all other members of the subgenus *Echinocotyle* it differs in possessing longer hooks. From *H. curiosa* (Szpotanska, 1931) Hughes, 1940 the present species differs in possessing testes in a triangle rather than a straight line. (Data from Szpotanska, 1931.) From *H. charadrii* Yamaguti, 1935 the present species differs in possessing longer hooks and testes in a triangle rather than a straight line, and in having spined suckers (data from Yamaguti, 1935, and Davies, 1939).

The present species closely resembles *H. laurei* Davies, 1939. The two species have hooks identical in size, shape, and number, and both are very small forms. In *H. crocethiae,* however, the testes are in a triangle rather than a straight line and the suckers are spined rather than unspined.

THE SUBDIVISIONS OF *Hymenolepis*

There have been several attempts to subdivide the unwieldy genus *Hymenolepis,* which contained 328 species in 1941 (Hughes, 1941). In extensive modern reviews of the genus, the following classifications were adopted:

Two subgenera (*Hymenolepis* and *Echinocotyle*)—Clerc (1902 and 1903); Ransom (1909); Fuhrmann (1906, 1908, 1924); Southwell (1930).

Three genera (*Hymenolepis, Wardium, Weinlandia*)—Mayhew (1925).

Four genera (*Hymenolepis, Wardium, Weinlandia, Fuhrmanniella*)—Tseng Shen (1932).

Three genera (*Hymenolepis, Echinocotyle,* and *Drepanidotaenia*)—Fuhrmann (1932); Joyeux and Baer (1936).

No subdivisions—Hughes (1940 and 1941).

Fuhrmann (1932) showed that the genera *Wardium* Mayhew, *Weinlandia* Mayhew, and *Fuhrmanniella* Tseng Shen were based upon characters (positions of the testes) variable in many species, and also impossible of delineation because nearly all possible positions of the testes within the segment are found in various species.

Drepanidotaenia Railliet was declared by Fuhrmann (1924) to lack characters for even subgeneric standing. Later Szpotanska (1931) redefined the genus to include only three species, one of which had an undescribed scolex. On this basis the genus was recognized by Fuhrmann (1932) and defined as follows: "Hymenolepidinés avec un petit scolex armé de 8 crochets. Strobila à cou très court et segments très large et courts. Glandes sexuelles femelles antiporales, les trois testicules du côté poral en une rangée transversale. Parasites des oiseaux." However, the included four forms have not a single character unique in *Hymenolepis* (*sensu latu*). Although the combination of characters listed by Fuhrmann is not found except in these four forms, a distinct genus is not set off. Numerous species (see Hughes, 1941), possess a small scolex and only 8 hooks, or very short neck and very wide, short segments. At least four species possess an aporal ovary and testes in a transversal row poral to the ovary (*H. ardeae* Fuhrmann, *H. bilateralis* von Linstow, *H. elongata* Fuhrmann, *H. przewalskii* Skrjabin). *H. crocethiae* Webster possesses an aporal ovary, but testes in a triangle. I regard *Drepanidotaenia* as defined by Fuhrmann (1932) as a valid subgenus.

Validity of *Echinocotyle* Blanchard as a subgenus was questioned by Mayhew (1925) and the characters and grouping not even mentioned by Davies (1939). Hughes (1940) refrained from an opinion. The group is an homogeneous one containing nine species (six listed by Fuhrmann, 1932, plus *H.* (*E.*) *multiglandularis* Baczynska and two species described in this paper). All have 10 hooks of slender shape, with guard rudimentary or lacking, and 1 or 2 accessory sacs, besides the unique character of suckers spined across the center. Otherwise, however, the included species seem to possess most of the variations found in *Hymenolepis;* I regard the group as a well-marked subgenus.

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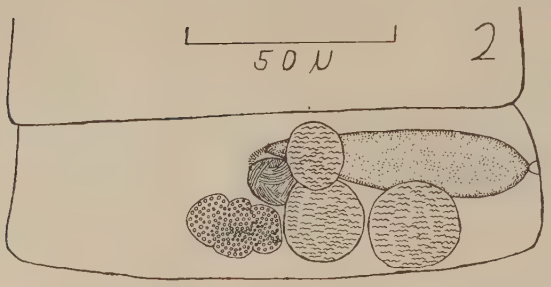
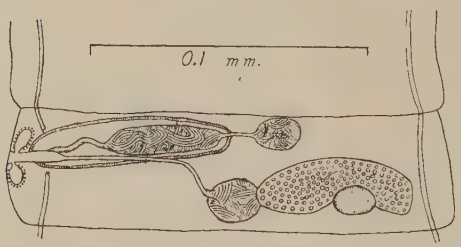
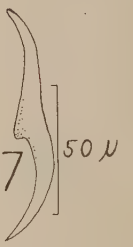
EXPLANATION OF PLATE I

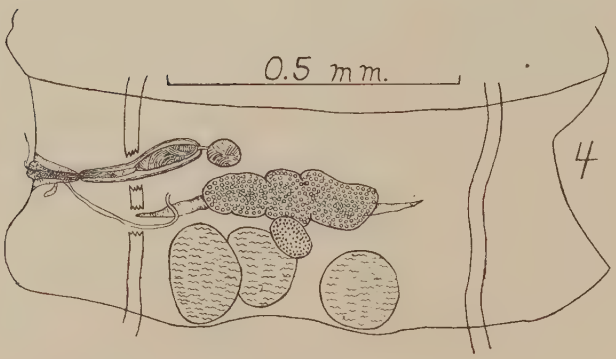
All drawings were made with the aid of a camera lucida

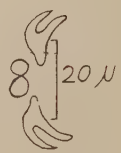
FIG. 2. *H. crocethiae* n. sp. Dorsal view of immature segment with developed testes.
FIG. 3. *H. crocethiae* n. sp. Ventral view of mature segment. The testes have disintegrated and are not discernible.
FIG. 4. *H. hughesi* n. sp. Ventral view of mature segment, with cirrus extruded.
FIG. 5. *H. hughesi* n. sp. Ventral view of partially gravid segment, only 15 segments posterior to Fig. 4, showing cirrus retracted.
FIG. 6. *H. crocethiae* n. sp. Rostellar hooks.
FIG. 7. *H. litoralis* n. sp. Rostellar hook.
FIG. 8. *H. hughesi* n. sp. Rostellar hooks.











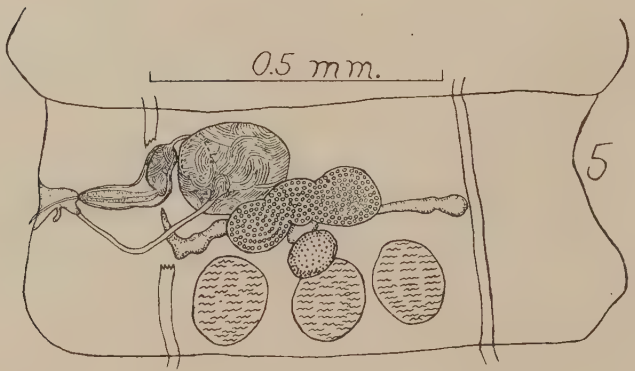


PLATE I

THE LIFE CYCLE OF *DIPHYLLOBOTHRIUM OBLONGATUM* THOMAS, A TAPEWORM OF GULLS*

LYELL J. THOMAS

This investigation deals primarily with the life cycle of a pseudophyllidean tapeworm infecting young gulls from Lake Michigan. It also examines the possibility of using larval characters as well as adult characters in establishing species. It is the writer's opinion that this is important at the present time when there is a tendency among taxonomists to use only adult characters in establishing species of the genus *Diphyllobothrium* with a resulting confusion of species.

METHODS

The tapeworm eggs for the following experiments were collected either from the feces of young gulls or by autopsy of fledgling gulls at the rookeries on Hat and Pismier Islands in Lake Michigan. The tapeworms were stored in Ringer's solution in a refrigerator and the eggs were teased out of segments as needed. By means of the refrigerator method the author has been able to keep viable eggs on hand at all times of the year.

For hatching purposes, eggs were repeatedly washed in distilled water and placed in covered Petri dishes. The eggs were stirred about from time to time with a medicine dropper but this did not prevent large numbers from being covered with bacteria and penetrated by fungi.

A paddle-wheel was devised to run by water power so that water in an 8″ × 12″ × 8″ aquarium was constantly stirred. The continual flow of water over the eggs kept them clean and produced better hatching. This same arrangement was used to adapt the first intermediate host, *Diaptomus oregonensis,* from Douglas Lake, to laboratory conditions. It was possible to rear parasite-free copepods in the laboratory by this means.

Coracidia were studied alive under the microscope, also as fixed and stained specimens. A drop or two of 2 per cent osmic acid was added to the water containing coracidia. After they had turned black they were dehydrated. When in 70 per cent alcohol a few drops of hydrogen peroxide were used to bleach the specimens. Alcoholic changes were made by allowing the larvae to settle in round bottom vials and the supernatant fluid was then drawn off by means of a medicine dropper. Other specimens in water were allowed to settle by evaporation upon albumen fixative on glass slides. Before complete dehydration could occur, killing was done by formaldehyde or osmic reagents. Heidenhain as well as Giemsa staining methods were used. A tannic acid mordant used with the Heidenhain stain and floxene counter stain produced black hooklets and nuclei with pink cytoplasm and cilia.

The procercoids were studied within the live *Diaptomus* or in Ringer's solution modified for CRUSTACEA.

Guppies, small viviparous fish, were used as second intermediate hosts because they were hardy and easy to handle.

Received for publication, January 2, 1945.

* Contribution from the University of Michigan Biological Station and the University of Illinois Zoology Laboratories.

Parasite-free herring gulls, *Larus argentatus* Coues. and ring-bill gulls, *Larus delawarensis* Ord. were reared from eggs collected in the rookeries. A home-made electric incubator at 103° F was used to hatch them. During their incubation the eggs were dipped in warm water two to three times daily. This seemed to be necessary to keep the shells from sticking to the chicks and to make most of them hatch. The newly hatched chicks were left in the incubator for the first twenty-four hours. They were then given their first feeding of herring with parasites. During the day they were placed where they had access to sunshine and at night were kept warm in a brooder. Cloacal temperature readings were recorded twice daily with a rectal clinical thermometer.

Infected herring, *Leucichthys artedi,* were obtained from the Bell Fish Company, Cheboygan, Michigan. The fish were taken from shoals either in the vicinity of Beaver Island or from the Straits of Mackinaw.

The writer wishes to express his appreciation to Doctors Penner and De Guisti for assistance in the rearing of birds during the course of the experiments and to all others who helped to make this work possible.

EARLY DEVELOPMENT OF *Diphyllobothrium oblongatum*

The ovoidal eggs of *D. oblongatum* (Fig. 11) average 61 μ by 39 μ. They are a light amber color with an operculum 19 μ in diameter and slightly tangential. The boss at the pole opposite to the operculum is obscure. They are undeveloped when laid. The ovum may be distinguished as a large opaque cell surrounded by darker granular vitelline cells. Development proceeds rather rapidly at temperatures ranging between 72° and 85° F. Eight days was the shortest recorded time for coracidia to be hatched from eggs shed from proglottids. One batch of eggs started July 18, 1938, in shallow water in Petri dishes had well-formed hooklets by July 23 and three days later were hatching. Yellowish granules of waste materials were left behind in the egg shells as the coracidia oozed out through the finely serrate opercular openings. The hooklets, which measure 11 μ in length, are at the posterior pole as the coracidium emerges. The coracidium rapidly elongates and contracts as it darts away but soon settles down to a smooth rolling motion as it becomes more spherical. In the elongated state its speed may be compared with that of the fluke miracidium. By increasing the salt content of the surrounding water it was possible to shrink the coracidium and to stop the ciliary action. The cilia would begin to beat and the embryophore would swell up as the salt content of the surrounding water was decreased. With increasing age of the coracidium, the cells of the embryophore take in water which finally causes a complete blocking of the ciliary action by the third day. These large spheres sink to the bottom of the container and only the onchosphere within remains active for a time.

Eggs taken from mature segments, July 9, 1939, were placed in distilled water and left in an electric refrigerator at a temperature near freezing. On August 21 the eggs were placed in an insulated quart jar and taken from the University of Michigan Biological Station to Champaign, Illinois. They were put undisturbed in a refrigerator at 3° C on August 22. Some of the eggs were removed to room temperature, 72° F, on November 15. By November 25 some of the coracidia were hatching. The onchospheres within were clearer than those observed hatching from fresh eggs. Eggs collected July 4, 1942, were stored in a refrigerator at about 3° C.

Some of them, on removal to room temperatures, hatched May 11, 1943; others, removed later, hatched on June 11, 1944. The cells of the onchospheres of these specimens were very transparent and the two flame cells were easily seen. This same transparency was observed by Thomas (1930a) in the onchospheres of *D. latum* in which the hatching of the coracidia was delayed under normal conditions. A possible explanation of this phenomenon is that the cells of the onchospheres have used up most of their food reserves.

On July 9, 1945, fresh gravid segments were quickly frozen solid in ice and left in the refrigerator in this condition until August 1, 1945. A few segments were thawed on that date and the eggs were teased out to incubate. Development progressed in these eggs and large numbers of coracidia began hatching on August 11, 1945.

There is every reason to believe that the same phenomenon will be found to be true for *D. latum* eggs. The chances for eggs to survive severe winter weather are greatly enhanced, thereby increasing the incidence and infective range of the parasite. Additional means for distributing the eggs were observed during an attempt to infect guppies with the plerocercoids of *D. oblongatum*. Eggs which had been added from time to time to the water in an aquarium were found on August 10, 15, and 26, 1939 to have passed unharmed through the intestines of the fish.

The eggs of *D. oblongatum* shed from proglottids hatch coracidia within 8 days to over two years, depending upon temperature. The onchospheres which develop in eggs over long periods of time at low temperatures are very transparent. Freezing eggs solid in ice for about a month did not destroy their ability to hatch normal coracidia.

DEVELOPMENT OF THE PROCERCOID IN THE FIRST INTERMEDIATE HOST, A COPEPOD

Numerous coracidia were given to *Diaptomus oregonensis* on July 24, 1938. A few infected animals with mature and partly developed procercoids (Fig. 1) were obtained on August 10. On July 9, 1939, conditioned *Diaptomus* were placed in an $8'' \times 12'' \times 8''$ aquarium and eggs of *D. oblongatum* were added. The paddle-wheel stirrer was maintained in operation throughout the experiment. By the end of July nauplii were abundant. Beginning July 20, samples of *Diaptomus* (Fig. 4) were taken at regular intervals and found to be infected. The copepods could be removed to Petri dishes for study without difficulty. Drawings were made of mature procercoids on July 31, 1939.

Later that fall, November 25, 1939, coracidia were placed with *Diaptomus oregonensis* from Lake Vermillion, Danville, Illinois. Numbers of mature procercoids were observed December 9 and some of the infected copepods which had been conditioned to the laboratory were carried in an 8-dram vial to the meeting of the American Society of Parasitologists at Columbus, Ohio, on December 26 and returned to the laboratory in good condition.

Procercoids were dissected out of copepods in Ringer's solution modified for CRUSTACEA on July 31, 1939. Each was surrounded by a delicate transparent envelop, similar to that which Vogel (1930: 638) has reported for the procercoid of *D. latum*, and possibly a chitinous secretion formed by the copepod. The anterior end of the larva was actively everted and inverted much like the proboscis of an acanthocephalan (Figs. 7, 8, and 10). Seven or eight alternating rows of large

retrorse spines were seen on the proboscis-like end (Fig. 9), followed by a thick coat of lesser and minute spines which covered the entire body down to the cercomer. The cercomer, containing the six hooklets, elongated then contracted into a sphere and was shed in the Ringer's solution. One typical procercoid measured as follows: cercomer 70 μ in diameter, body proper 219 μ long and 87 μ wide at widest part. The cercomer was observed to move about for some time after it was shed. Another procercoid measured 202 μ in length fully entended and about 65 μ at the widest part. The base of the proboscis-like end measured about 39 μ in width. The cercomer extended was 30 μ in diameter. Within the body proper, a dense glandular region was seen with a duct opening at the distal end of the proboscis. A viscous, glandular substance oozed from the pore (Fig. 9). This secretion appeared to be similar to that from the histolytic glands of cercariae. Excretory ducts and a few flame cells were observed outside the glandular area, together with a small number of calcareous bodies.

The procercoid develops to the infective stage in the body cavity of *Diaptomus oregonensis* in two weeks or more depending upon temperature. The eversible proboscis has 7 to 8 rows of large retrose spines. In the copepod the procercoid is covered with a delicate transparent envelop, possible chitinous in origin. Heavy internal glands secrete a histolytic-like substance through a duct and pore at the anterior end.

DEVELOPMENT OF THE PLEROCERCOID IN THE SECOND INTERMEDIATE HOST, A FISH

Four dozen parasite-free guppies were placed in an 8″ × 12″ × 8″ aquarium containing infected *Diaptomus* August 5, 1939. Five days later three of the fish were dissected and found to contain plerocercoids in the stomach walls and mesenteries. Little change was noted in these minute parasites except that they had become very opaque. The infected fish were returned to the University of Illinois where many of them died within a ten day period, due apparently to too heavy an infection.

A dozen remained, however, and were examined from time to time. The plerocercoid after about two months (Fig. 2) was approaching the youngest stages found in natural infections in herring. The last fish examined on December 18, 1939, had one plerocercoid which measured 1.193 mm long and 0.325 mm wide. Bothria had appeared and spines still covered the body. This plerocercoid was similar to those obtained from a naturally infected minnow, *Notropis deliciosus* and from ciscos, *Leucichthys artedi,* from Douglas Lake. A protective wall was developed by the host tissue around the parasite to form a cyst.

Typical plerocercoids were formed in guppies 135 days after the fish had been fed infected copepods.

INFECTION OF THE FINAL HOST, THE GULL

Beginning July 20, 1938, three fully feathered young herring gulls were fed daily for one week with fresh herring containing numerous stomach cysts of *Diphyllobothrium* sp. Daily examinations were made after the second week without finding eggs in the feces and no tapeworms were present when they were killed on August 3.

Two herring gulls partly covered with down were given two stomach cysts each on July 23, 1938. They were autopsied two weeks later and no tapeworms were found. Another bird partly feathered was fed daily on fresh stomach cysts beginning August 2. One *Diphyllobothrium* plerocercoid was found in the lower

intestine, apparently on its way out. A young ring-bill gull about one week old was fed numerous cysts August 3, 1938. Two weeks later the bird was killed and one immature *Diphyllobothrium* was found in the small intestine.

Four herring gulls, about two weeks old, were secured from the Hat Island, Lake Michigan, rookery June 30, 1939. They were given four cysts each from the stomachs of fresh herring on July 3. The birds were fed cooked fish until autopsied July 16. Previous to this date no eggs were found in the feces and no worms were present at autopsy.

Four young herring gulls hatched in the laboratory July 15, 1939, were fed daily with stomach cysts from herring for one week. On August 13 the birds were autopsied and one immature *Diphyllobothrium* was taken from each.

A young herring gull, partly feathered, was obtained from Hat Island rookery on July 15, 1939. Eggs appeared in its feces the first week of captivity, and, on July 27, proglottids of *D. oblongatum* appeared in its feces. On August 4 the rest of the worm was shed. This was a natural infection.

It was now evident that age immunity, species immunity, and body temperature were all factors to be considered in establishing the infection in young gulls.

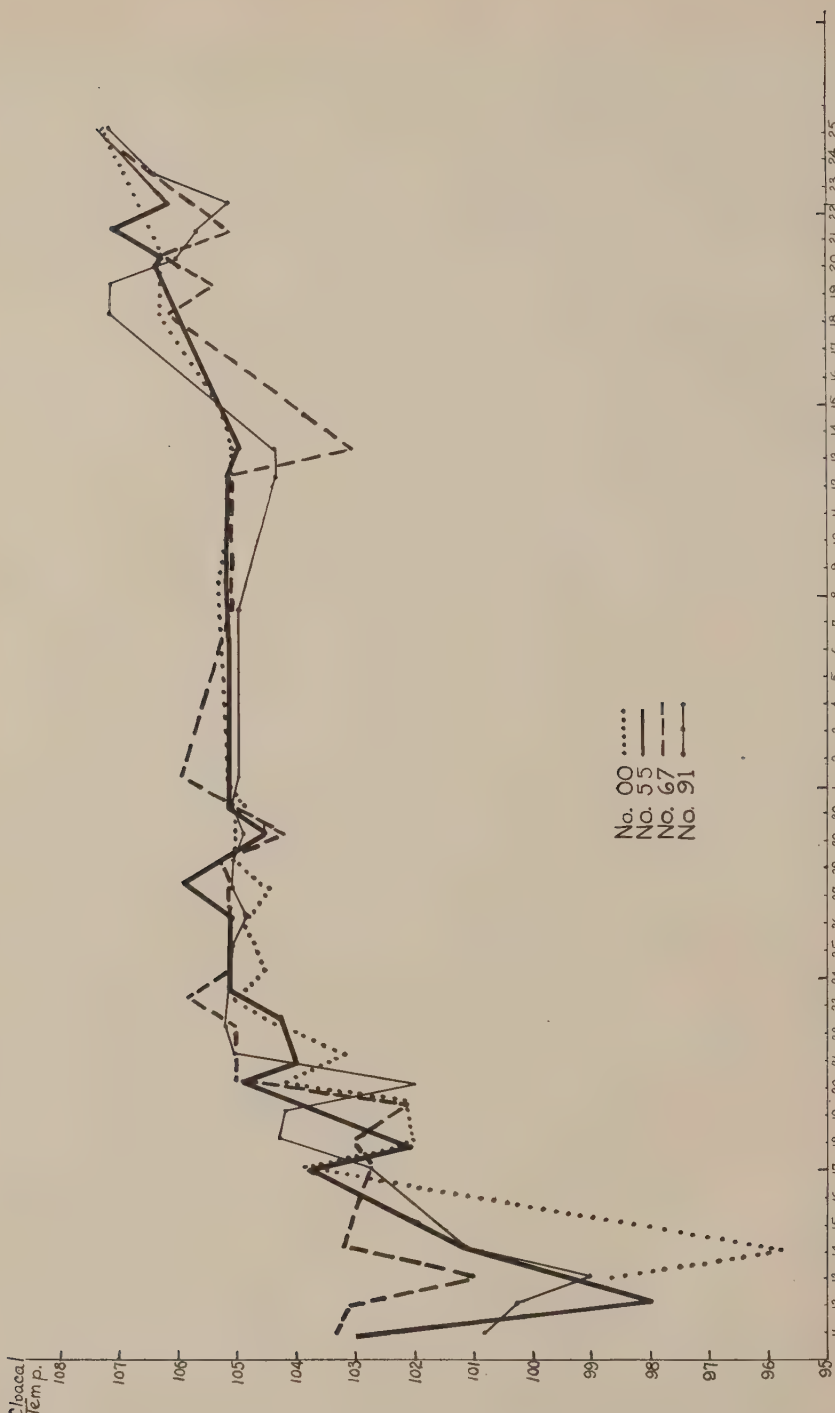
Six eggs of ring-bill gulls were obtained from Pismier Island rookery July 4, 1940. Twenty-four hours after hatching each of four birds was given at least four stomach cysts daily. Thereafter fish were fed without regard to the numbers of cysts present. Daily cloacal temperatures were taken morning and evening with the average temperature readings shown in Text Figure No. 1. During the first week, the cloacal temperatures fluctuated exceedingly. By the middle of the second week the temperatures began to level off between 103° and 105° F until the fourth week. Then, after a slight drop during the fifth week, they rose to a peak of about 107° F. After another slight drop they were mounting again when autopsied August 25. At the end of the experiment the birds were fully feathered and able to fly. Their cloacal temperatures were approaching the temperature given by Wetmore (1921) for adult herring gulls. He shot the birds and took the temperature readings as soon as possible afterwards, and recorded the mean temperature as 108.2° F.

Eggs were observed in the feces of gull No. 67 after 24 days; gull No. 55 after 25 days; gull No. 91 after 29 days; and in gull No. 00 after 26 days. Shortly after this, worm segments began to appear in the feces and when the birds were autopsied August 25, no tapeworms were present.

Young gulls can be infected during the first week after hatching when their cloacal temperatures are low. Tapeworms of the genus *Diphyllobothrium* are expelled from young gulls after maturity is reached in 3½ to 4 weeks. During the growth of both the tapeworms and the young gulls the cloacal temperatures of the gulls rise from below 100° to over 107° F. After the initial infection, additional feedings of cysts do not increase the worm burden.

SUMMARY OF THE LIFE CYCLE OF *D. oblongatum*

1. Eggs from shed proglottids hatch coracidia in 6 days to two or more weeks depending upon temperature.
2. Freezing of eggs solid in ice does not destroy their ability to develop and hatch.
3. Developing eggs may pass through the intestines of guppies unharmed.
4. Coracidia may hatch from eggs kept two or more years in a refrigerator.



TEXT FIGURE No. 1. The lines record the average cloacal temperatures of ring-bill gulls from July 11 to August 25, 1940. See text for dates when eggs of *D. oblongatum* first appeared in the feces.

5. The procercoid develops to the infective stage in *Diaptomus oregonensis* in two weeks or more depending upon temperature.

6. Typical plerocercoid cysts formed in guppies in 135 days after the fish had fed upon infected copepods.

7. Similar cysts from natural infections in ciscos, *Leucichthys artedi,* fed to laboratory-reared herring gulls, *Larus argentatus,* and to ring-bill gulls, *Larus delawarensis,* developed to adult worms in three and a half to four weeks, after which they were shed.

DISCUSSION

The general pattern of development in *Diphyllobothrium oblongatum* is the same as that given by Jánicki and Rosen (1917) for *D. latum.* Thomas (1946) has shown variations between adult worms of *D. oblongatum* and *D. latum.* Although Skinker (1931 and 1932) could not find enough differences to separate *D. cordiceps* from *D. latum* the writer is of the opinion that a more careful check on both adult and larval characters will show sufficient differences. In *D. oblongatum* the eggs have the operculum at a slight tangent and the boss is obscure. This is not true in the case of *D. latum.* Nicholson (1932: 169) stated, regarding low temperatures and their effects on the eggs of *D. latum,* "During the winter, low temperatures kill ova deposited on excreta on ice." He further stated that the water is too cold for any of the embryos to hatch from eggs that might be brought in by running streams or sewage. This is certainly not true for the eggs of *D. oblongatum* in which the freezing solid in ice and low temperatures for over two years do not prevent their hatching. Nicholson (1932) did not state that the eggs of *D. latum* were examined under experimental conditions by freezing them and holding them at low temperatures.

The hooklets of the onchosphere in *D. oblongatum* are 11 μ long. In another species of *Diphyllobothrium* from gulls the hooklets measure only 8 μ in length. In other families and genera of the PSEUDOPHYLLIDEA the lengths of hooklets differ. For example, in *Haplobothrium globuliforme,* Thomas (1930b) found that they measure 9 μ in length. In one *Ligula sp.* they measure 13 μ in length.

The cilia in the coracidia of different families and genera of PSEUDOPHYLLIDEA vary as to length and type of movements. The character of the pellicle-like covering of the embryophores differs. In *Haplobothrium* the cilia are shorter and more numerous than in either *D. oblongatum* or *D. latum.* The coracidium of *Bothriocephalus rarus* Thomas (1937) has more or less of a jiggling movement. This was not true of coracidia of any *Diphyllobothrium* thus far observed. Vogel (1929: 217, fig. 2), by means of dark-field illumination, has demonstrated that in *D. latum* the cilia are definitely longer at the pole opposite the hooklets. Rosen (1919: 269, fig. 1) reported the cilia of *Triaenophorus nodulosus* to be five times as long at one pole as at the opposite one.

The procercoid of *D. oblongatum* is provided with 7–8 alternate rows of heavy retrorse spines at the anterior proboscis-like end. These are followed by a thick coat of very minute spines which cover the body proper down to the cercomer. The spines together with the histolytic-like glandular secretions would furnish ample means for penetrating the gut wall of the fish host and in an early infection should allow transfer to a second intermediate fish host. The spines are retained for sometime in the plerocercoid stage. Wardle (1933) reported that in the plerocercoid of

D. latum there appears to be "no mechanism which would enable it to bore through the alimentary tract of the large fish and penetrate into the muscles. In fact there appears to be no grounds whatever for assuming that the plerocercoid stages in the muscle tissues of a fish can pass from the gut of a second fish and into its musculature." The writer plans to test this possibility by experiments with *D. latum* and *D. oblongatum*.

In *Haplobothrium* no spines are evident in the procercoid and penetration seems to be accomplished by means of histolytic glands. The plerocercoid develops as a cyst in the liver of the second intermediate host, a fish. *Bothriocephalus rarus* develops bothria in the procercoid stage and has no provision for penetration as it never leaves the gut of the newt following the ingestion of the infected copepod. Li (1929: Pl. XIV, figs. 26 and 27) reported about 12 rows of hook-like spines at the anterior end in the procercoid of *Diphyllobothrium decipiens*. They appear to be much like the hooklets of the onchosphere. In fact, they are different from those observed by the author in *D. oblongatum* and in those indicated for *D. latum* by Janicki and Rosen (1917) and by Vogel (1930: 638, fig. 7).

The writer was of the opinion (Thomas, 1938 and 1939) that the plerocercoids in stomach cysts from herring in Lake Michigan were those of a tapeworm infecting both birds and mammals. Careful studies since then have revealed that they are different species. There are several species infecting birds. *D. laruei* from the dog (Vergeer, 1942), and one from cats (unpublished work of the writer) are separate species. Although Vergeer (1942) believed the cysts of *D. laruei* to be generally distributed throughout the Great Lakes Region, he obtained it with great difficulty over an extended period of feeding experiments. The writer after feeding similar cysts to cats for nearly ten summers has obtained the infection only three times.

Scott (1935) stated, "*Diphyllobothrium cordiceps* is found in pelicans, gulls, and both species of bears; however, it is less thrifty and not so large in gulls, and in bears it is probably always sterile." He suggested a hybridization is indicated here between *D. cordiceps* and *D. cordatum*. Skinker (1931 and 1932) could not find sufficient characters to separate these two worms from *D. latum*. Vogel (1929) considered burbot livers as the source of a heavy infection of the population in East Prussia because of their habit of spreading the raw fish livers with salt and pepper on bread. The question is, are these cysts of *D. latum* or some other species of *Diphyllobothrium?* Vergeer (1944) believed some of these cysts in the liver to be those of *D. latum*. Similar cysts occur in the *Lota maculosa* or burbot of the Great Lakes. The writer still awaits experimental proof to establish these liver cysts as those of *D. latum*. Hickey (1944) and Lapage (1945) were of the opinion that *D. ditremum* from gulls and cormorants (as determined by Baylis) was also capable of infecting rats and puppies. Unsworth (1944) was reluctant but inclined to hold this same view. Järvi (1909) took for granted that the stomach cysts in *Coregonus albula* from Finland were those of *D. latum*. Simms and Shaw (1931) believed the plerocercoid cysts on the stomachs and mesenteries of the trout in Elk Lake, Oregon, to be those of *D. cordiceps*. The determinations, according to them, were made by means of plerocercoids and immature worms.

Unfortunately there is not as yet enough information available whereby one can positively identify the species of the genus *Diphyllobothrium* from the plerocercoids. Experimental evidence is too insufficient to suggest that the control of the gulls

would solve the infection in trout. Until we definitely know the differences, if any, which exist in these larval stages of each species we cannot correctly identify the species from plerocercoids. This conclusion is reached by a study of the adult as well as the larval stages of only one species. We predict that if the same study is made of all species, specific differences will be found.

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Description of Plate

All drawings unless otherwise designated were made with the aid of a camera lucida. The projected scale to the left of figures represents for Figs. 1, 4, and 14, 0.10 mm; for figs. 2, 3, 5, 6, 11, 12, 13, and 15, 0.02 mm; for figs. 7, 8, 9, and 10, 0.05 mm.

Fig. 1. Different stages of procercoids in *Diaptomus* infected with coracidia of *Diphyllobothrium oblongatum* on July 24, 1938, and examined Aug. 10, 1938.

Fig. 2. Plerocercoid of *D. oblongatum* removed from stomach cyst of experimental guppie two months after the infection.

Fig. 3. Coracidium of *D. oblongatum* several minutes after hatching.

Fig. 4. Onchospheres newly penetrated into haemocoel of *Diaptomus* placed with undeveloped eggs, July 9, 1939, and examined July 20, 1939.

Fig. 5. Eggshell with operculum open and a few waste granules remaining within the shell.

Fig. 6. Egg with fully developed coracidium ready to hatch after two weeks development.

Figs. 7 and 10. Procercoids in Ringer's solution for Crustaca showing stages of movements in proboscis and cercomer.

Fig. 8. Procercoid with fully extended proboscis; the cercomer was shed in Ringer's solution.

Fig. 9. Proboscis of procercoid under oil immersion showing large spines and secretion droplets.

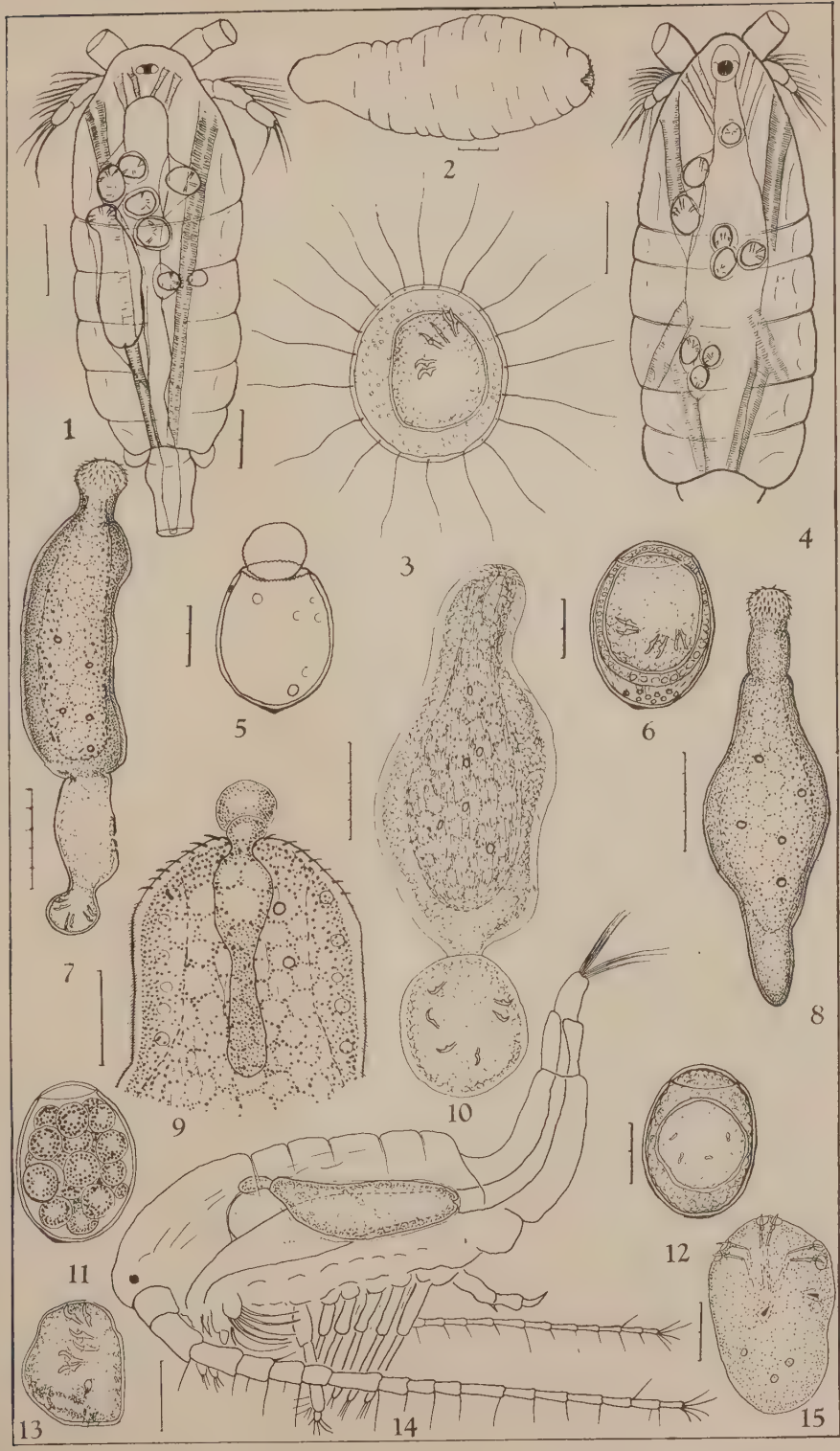
Fig. 11. Undeveloped egg newly laid.

Fig. 12. Egg after ten days of development.

Fig. 13. Onchosphere released from embryophore, one flame cell showing.

Fig. 14. Two mature procercoids in *Diaptomus* after about 10 days development.

Fig. 15. Young procercoid removed from *Diaptomus* one week after infection; muscle fibers, flame cells, and calcareous bodies evident.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A CRITICAL REVIEW OF TERMINOLOGY FOR IMMATURE STAGES IN ACANTHOCEPHALAN LIFE HISTORIES

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Prior to 1935 there had been no definite names proposed for the stages in the ontogeny of the ACANTHOCEPHALA. Up to that date immature stages had been referred to merely as "larval acanthocephalans" without recognition of the fact that at least two distinctive types of larvae are readily recognizable. In 1935, in a very brief abstract, the present writer suggested two terms, acanthor and acanthella, as a contribution toward permitting more precise reference to developmental stages in members of this group of worms. Detailed descriptions of these two stages were not published until 1937. In a relatively short time the two names have come into very general use in textbooks as well as in the special literature. It seems possible that lack of general accessibility of the second paper (Van Cleave, 1937, in the Skrjabin Jubilee Volume, Moscow) may have led to acceptance of the terms without precise understanding of their connotation. Rather conspicuous misapplication of these terms in recent literature seems to call for a note of correction. This appears particularly important since among investigators of the Acanthocephala there seems to be a very general misunderstanding of the terms "larva" and "metamorphosis" as they are applied in technical biological literature.

Anton Meyer (1928 et seq) was one of the first recent students of ontogeny of the ACANTHOCEPHALA to misconstrue the distinctions between larval and juvenile worms. His work on the life history of *Macracanthorhynchus hirudinaceus* was published before the terms acanthor and acanthella were introduced, hence he followed the practice of earlier workers, referring to the stages in the arthropod host as "larva" and "juvenile." In his discussion and in legends of his illustrations of developmental stages he referred to the newly hatched individual (the acanthor) as a larva, but he used the term "juvenile" to apply to all later stages of development within the arthropod host. He thus failed to recognize the fact that the series of stages following the acanthor, before the proboscis becomes functional and differentiation of tissues has set in, are likewise larval. This seems especially strange since Meyer's observations on cytological changes accompanying transformation of the acanthella to the juvenile are among the best available evidences that a true metamorphosis is here involved.

Throughout the present discussion, the concepts of "larva" and "metamorphosis" are those generally accepted in technical zoological literature. In popular usage, metamorphosis applies to any conspicuous change in the form of an individual subsequent to the early stages of embryonic development. Among investigators who use the term in a more precise sense this transformation involves more than the simple attainment of functional reproductive organs from the rudiments developing progressively in the larvae. It correlatively implies the loss to the adult of at least some organs or structures distinctive of the larva. In the transformation of an acanthocephalan, the rostellar apparatus of the acanthor is wholly lost in later stages and hence one of the most distinctive organs of the larva is lost in metamorphosis. Stu-

Received for publication, February 10, 1947.

dents who are not familiar with metamorphosis in its varied aspects are inclined to place too much emphasis upon the external transformation in shape of body when in reality cytological and histological changes are much more significant than the gross external evidences of change.

In 1928 the present writer called attention to the fact that in all but a few families of ACANTHOCEPHALA (EOACANTHOCEPHALA) there is conspicuous transformation of the cells of the somatic tissues accompanying metamorphosis. The truly embryonic cells of the earliest stages in ontogeny contain large spheroidal nuclei which are retained as "giant nuclei" by several of the tissues (lemnisci and subcuticula especially) throughout life of the individual in the EOACANTHOCEPHALA. Similar large nuclei are distinctive in many of the structures of all acanthellas that have been studied. In later development of forms other than EOACANTHOCEPHALA these "embryonic nuclei" undergo changes which transform them into the nuclei of the functional adult tissues. Meyer (1938: 177, figs. 28, 29) has shown graphically how the unspecialized nuclei of the body wall in late acanthella stage of *Macracanthorhynchus hirudinaceus* become rearranged and changed in form in the juvenile worm. This change in form and arrangement of the nuclei is attained at the same time that the surrounding cytoplasm becomes differentiated to form the subcuticula with its distinctive fibers and lacunar vessels which were wholly lacking in the acanthella. It is on evidences such as these that the present writer considers the acanthella as a larval stage and regards the juvenile worm with its metamorphosed organs and tissues as a product of metamorphosis.

The present writer has been unable to understand the basis on which Lundström (1942) made the empirical statement that there is no metamorphosis in the ACANTHOCEPHALA. Lundström accepted the names acanthor and acanthella for developmental stages but he apparently did not consider either of these stages as "larval." In his discussion of the life history (page 138), he proposed that the term "larva" be restricted to the free or encysted individuals occurring in the body cavity of a vertebrate other than the definitive host. This stage is obviously the juvenile which has attained a functional proboscis and at least all the rudiments of the internal organs including the reproductive system. Such juveniles are not in any fundamental respect different from the juvenile that occurs in the arthropod host after transformation of the acanthella. They may be somewhat larger than the forms in the arthropod and in some species the shape of the body changes conspicuously but these are only the result of growth processes. Furthermore, Lundström's restriction of these free or encysted individuals in the body cavity to occurrence in a vertebrate *other than* the definitive host is in error because in a number of instances (various species of *Echinorhynchus, Leptorhynchoides, Neoechinorhynchus, Pomphorhynchus*, and many other genera) the same definitive vertebrate host individual may shelter mature worms in the intestine and carry juveniles of the same species in the body cavity or in visceral cysts. This is particularly true of cannibalistic species of host which through their feeding habit become both second intermediate host and definitive host to the same species of acanthocephalan.

In a series of two papers, Donald V. Moore (1946, J. Parasitol. 32(3): 257–269 and 32(4): 387–399) has presented results of experimental studies on the life histories of *Moniliformis dubius* Meyer, 1932 and *Macracanthorhynchus ingens* (von Linstow, 1879). The former of these species reaches sexual maturity in rats and

utilizes the American cockroach (*Periplaneta americana*) as its arthropod intermediate host. The latter parasitizes the raccoon (*Procyon lotor lotor*) after passing its larval development in scarabaeid larvae; *Phyllophaga crinata* Burmeister, *P. hirtiventris* Horn, and *Ligyrus sp.* having been experimentally determined as suitable arthropod hosts. In both of these papers, Moore has adopted the terminology proposed by the present writer as names for developmental stages of ACANTHOCEPHALA. However, he has applied one name to a stage entirely different from that for which it was originally intended. The name acanthor he has used correctly throughout his publications for the first larval form which normally emerges from the embryonic membranes only after the acanthocephalan egg has been swallowed by the arthropod host. In describing and naming the later stages in development in both of his papers, Moore has seen fit to reassign the name acanthella to the fully transformed juvenile stage of the worm. Thereafter he proceeded to coin a new term, "preacanthella," for the series of transitional stages between acanthor and infective juvenile. His preacanthella stages are in reality the acanthella stages of earlier writers.

This intentional shift of the name acanthella from a truly larval form to a juvenile state does violence to the original concept which was expressed in the following words (Van Cleave, 1935: 436): "The name acanthella is here proposed for this series of stages extending between the acanthor and the fully formed worm ready to infest the final host." Later (Van Cleave, 1937: 741), this statement was expanded to the following form: "The term acanthella is here proposed to designate the series of immature stages of an acanthocephalan (Figs. 3 to 6) which develop progressively within the body of the invertebrate host. In this series of stages the definitive organization of the mature worm is being laid down. The proboscis is non-functional in the acanthella and the rostellar spines characteristic of the acanthor of many species become lost as do the spines of the transformed hexacanth larva of Cestoda."

From the foregoing quotations the original concept of the acanthella as a series of larval stages is stated in unmistakable terms, hence the proposed use of the term for a post-larval stage which has already undergone metamorphosis seems wholly unwarranted. It should be noted that in the original characterization of the acanthella the lack of a fully formed, functional proboscis is mentioned along with the recognition of a progressive series of stages immediately following the acanthor stage. In the light of detailed studies on life histories such as those conducted by Meyer (1933, 1938), by Kates (1943), and by Moore (1946), it is now obvious that the cytological changes accompanying metamorphosis of the acanthella to the juvenile stage are more significant as evidence of metamorphosis (in species that have been investigated) than the attainment of a functional proboscis as originally maintained. Kates (1943, figs. 7, 8) has shown that a proboscis is present in the late acanthella (acanthella stages IV and V of Kates) before the larval tissues have undergone metamorphosis. The juvenile stage to which Moore applied the term acanthella is in reality not a larval stage at all but is a post-larval juvenile form which has completed its metamorphosis. The juvenile has lost the morphological and cytological features distinctive of the larva and has attained the rudiments of the organs characteristic of the adult worm, thus satisfying the generally accepted definition of metamorphosis.

As a corollary to his misuse of the term acanthella for the juvenile worm, Moore (1946) has introduced as a new name the term pre-acanthellas for the series of

progressive stages originally designated as acanthellas. It has long been recognized that a succession of stages intervene between the newly hatched acanthor and the juvenile worm. In this series, clear-cut distinctions between the successive forms are lacking since one stage passes gradually and insensibly into the next without any recognizable time interval or static condition between them and without any change of position or radical alteration that would mark a definite limit to a fixed stage.

Even in the development of nematodes and of the arthropods, where each successive stage is marked by a molt which sharply divides the larval existence into a series of distinct instars, the term "larva" is applied to all of these instars collectively. In the ACANTHOCEPHALA, for convenience of description of the successive growth stages of the acanthella, gradations as fine as may seem desirable may be recognized in descriptive morphological studies. Thus Kates (1943), in describing the development of *Macracanthorhynchus hirudinaceus* in the intermediate host, found it convenient to recognize two stages of the acanthor and six different stages in the acanthella of that species. He referred to the latter as acanthella, stage I; stage II; etc. Acanthella stage VI he termed the "infective acanthella." Since this stage has acquired a functional proboscis, capable of introversion and the individuals are "essentially juvenile parasites possessing the structures and organ systems of the adult worms" the present writer maintains that transformation has been completed and the young worm of this "acanthella stage VI" of Kates is no longer a larva but is a juvenile worm.

There have been but a few species of ACANTHOCEPHALA for which details of development have been published. To the present time most of the species on which detailed information is available belong to the ARCHIACANTHOCEPHALA. There is no necessary assurance that in representatives of the EOACANTHOCEPHALA and the PALAEACANTHOCEPHALA the pattern of development is identical. It seems probable that in different genera the rate of the transformation and the time involved might result in lengthening or shortening the series of stages recognizable in the acanthella stage.

In his Introduction to Parasitology, Pearse (1942: 125) has correctly described the development of an acanthocephalan but his fig. 162, portraying the life cycle of *Macracanthorhynchus hirudinaceus,* is very seriously in error in labelling and in arrangement of the stages. The acanthor is mislabelled as acanthella and is misplaced in the cycle immediately preceding the adult worm instead of between the egg and the true acanthella.

Because of the very general misunderstanding of the details of the life cycle of an acanthocephalan as revealed in the foregoing review of recent literature, it seems desirable to present the information in the form of a chart. In almost all of the literature the individual stages in the developmental cycle of *Macracanthorhynchus hirudinaceus* were presented in diverse magnifications that permit of no immediate appreciation of the relative sizes of the individual stages. Meyer (1931: 170) presented a schematic diagram of the stages that occur in the beetle larva but they are lacking in the intimate detail of morphological structure which Meyer (1932–1938) and Kates (1943) worked out for the developmental stages of this species. In the illustrations accompanying the present article detailed morphological drawings presented by Kates (1943) have been redrawn to a uniform magnification (Plate I)

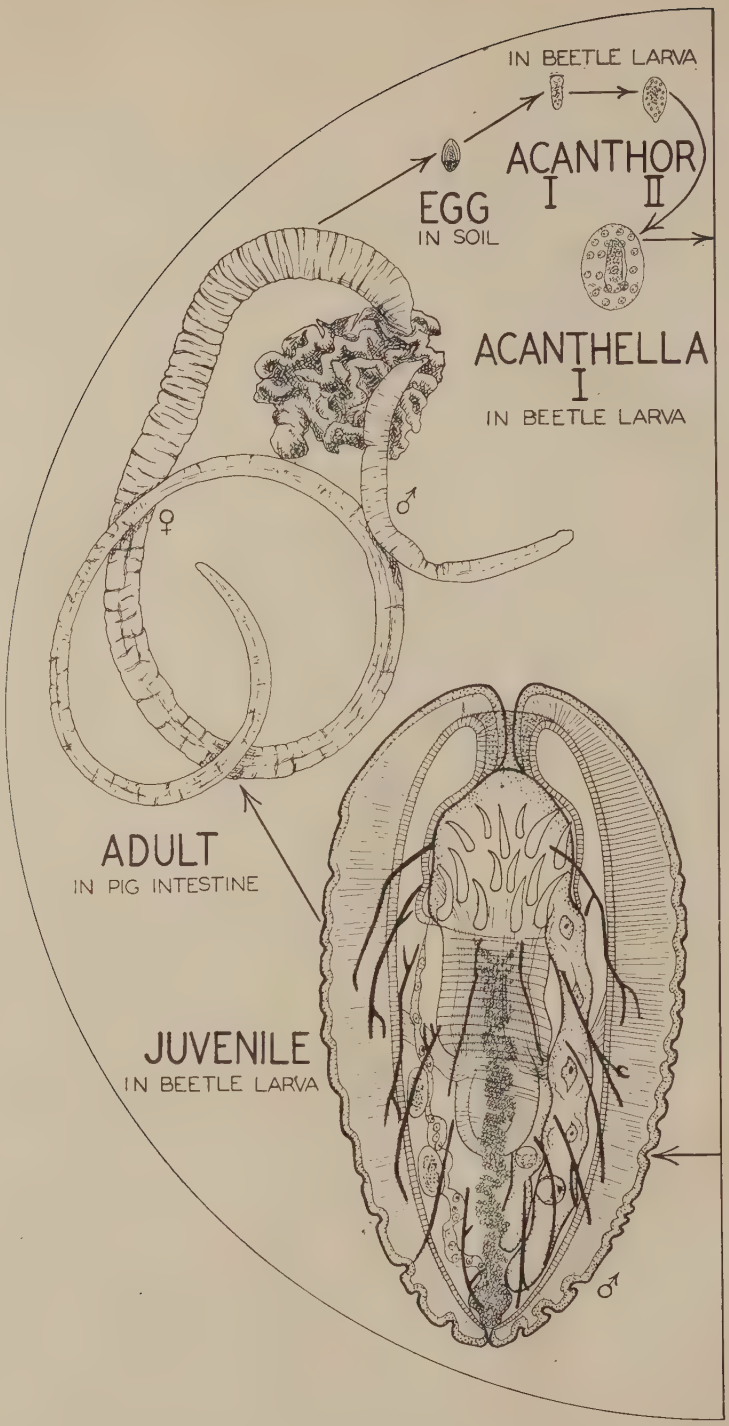

PLATE I

The life cycle of *Macracanthorhynchus hirudinaceus* (Pallas), the thorny-headed worm of swine. Drawings of adult worms about natural size; all other stages at fairly uniform magnifi-

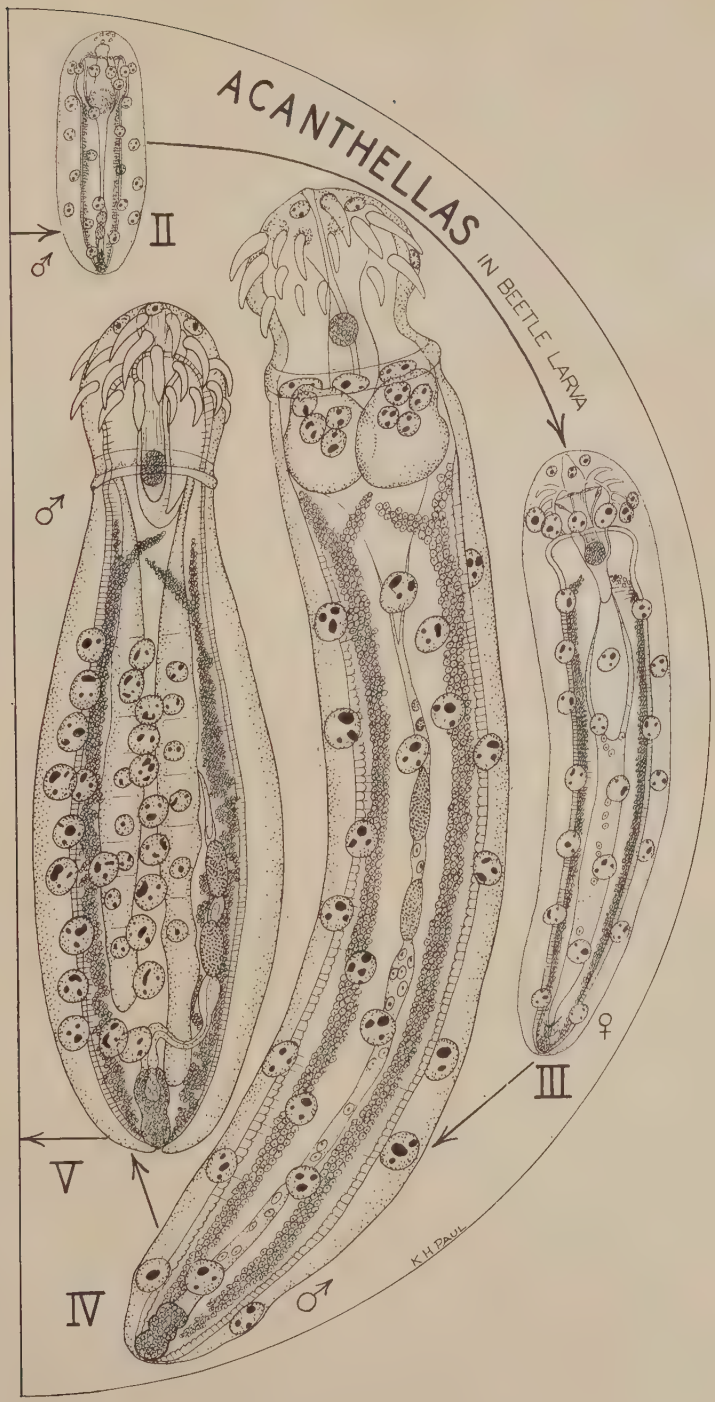

PLATE I

cation of approximately 38 diameters. All figures, except those of adults redrawn and adapted from Kates (1943). See text figure 1 for details of egg and acanthor. Plate arranged and drawn by Katherine Hill Paul.

and to these is added a drawing, approximately natural size, of the male and female of *M. hirudinaceus* to make the cycle complete. Because the egg and the acanthor in this plate are so relatively small these stages have been redrawn (Text figure 1) at a uniform magnification sufficient to show full details that are lacking in the plate.

By way of summary, in typical ontogeny of the ACANTHOCEPHALA (see Plate I), the fertilized egg within the body cavity of the female worm develops into an embryo surrounded by a series of membranes or shells. In subsequent development the embryo within these membranes is recognizable as a distinct larval form, the acanthor, which normally hatches from its confining membranes only when the egg is swallowed by a suitable species of arthropod. In the gut of the arthropod host the rostellar spines of the acanthor (Plate I and text figure 1, B), which have no direct relation-

TEXT FIGURE 1. Shelled embryo and acanthor stages of *Macracanthorhynchus hirudinaceus* (Pallas) to show details not available at magnification adopted for life cycle plate. Redrawn from Kates (1943). Magnification approximately 500 diameters. A. "Egg" or shelled embryo. Surface appearance shown only on lower pole; on remainder of drawing membranes are shown in optical section. B. Acanthor, stage I, from lumen of mid gut of beetle larva. Note the larval rostellar hooks and small body spines. C. Acanthor, stage II, after penetrating the wall of the mid gut of the beetle larva, about 5 to 20 days after artificial infection.

ship with the proboscis hooks of the adult worm, bore through the wall of the digestive tube of the host. For a time the chief features of the acanthor may still be recognizable after penetration of the gut is accomplished. This stage (text figure 1, C) Kates (1943) has termed the second stage of the acanthor. Within the body cavity of this host, the acanthor undergoes conspicuous metamorphosis, without intervention of any reproductive cycle. Each acanthor transforms into a lowly organized ball of embryonic cells. This is the initial stage (Acanthella I) in a succession of larval stages called the acanthellas. Gradually and without sharply recognizable instars, (Acanthellas II to V) the rudiments of the structures of the adult worm make their appearance. As a final step in development (Juvenile) within the arthropod body, rudiments of the organs and structures of the acanthella become transformed into the structures distinctive of the adult worm. The proboscis and its

hooks become functional and the embryonic cells become differentiated into the respective tissues of the adult. With the appearance of these features of the mature worm, the larval existence is terminated and the individual is recognizable as an immature acanthocephalan to which the term juvenile is appropriately applied.

Further development to sexual maturity may follow the ingestion of the infected arthropod by a suitable vertebrate host in whose intestine growth and full bodily development of the juvenile proceed. In many species of ACANTHOCEPHALA, the arthropod bearing acanthellas or juveniles may be eaten by an intermediate host, either vertebrate or invertebrate, in whose body cavity the juvenile lies free or becomes secondarily encysted. With the intercalation of the second intermediate host or reservoir host into the life cycle, the worms may attain sexual maturity only when this second intermediate host is eaten by a proper definitive host.

Failure to recognize a distinction between larval forms and metamorphosed juveniles in the parasitic worms may seem to be relatively unimportant, resting wholly upon definition of terms. Serious misinterpretations of biological principles has often resulted from incorrect use of technical terms. A generation ago workers on the life histories of digenetic trematodes regarded the entire succession of stages occurring in the snail host, as "larval trematodes." It was only with realization of the fact that sporocysts and rediae are as truly "adult" as the marital stage within the definitive host that the full significance of the trematode life history became generally understood. In the ACANTHOCEPHALA there is no reproductive cycle within the arthropod host, but biologically it becomes of distinct importance to recognize the point in the life cycle at which the larva undergoes metamorphosis to the fundamental form of the adult worm.

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ON THE OCCURRENCE OF THE ACANTHOCEPHALAN GENUS *TELOSENTIS* IN NORTH AMERICA

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In 1892, Edwin Linton recorded what he considered to be *Echinorhynchus pristis* and a variety of that same species which he named *E. pristis tenuicornis* from marine fishes of New England. All of the specimens which he identified as *E. pristis* were incorrectly named. There has never been an authenticated record of this species, which is now recognized as *Rhadinorhynchus pristis,* from the American continent. In 1918, the present writer attempted to establish the status of these forms which Linton regarded as *"E. pristis."* He accepted the description which Linton had given for *E. pristis* as the basis for the recognition of a distinct species to which the name *Rhadinorhynchus ornatus* Van Cleave was applied. All later studies have confirmed the validity of the species *R. ornatus* although its generic assignment was later changed when the old concept of *Rhadinorhynchus* was shown to contain several clearly distinguishable groups. Chandler (1934) was one of the first investigators to lay the foundations for recognition of a number of genera retrieved from the heterogeneous group of species that had been assigned to *Rhadinorhynchus.*

Chandler tentatively assigned *R. ornatus* to the genus *Nipporhynchus* for which he designated *N. katsuwonis* (Harada, 1928) as genotype. The present writer (in Van Cleave and Lincicome, 1940) declared *N. katsuwonis* to be a direct synonym of *Nipporhynchus ornatus* and thereby the last named species became type of genus *Nipporhynchus.* The confusion in the literature resulting from the misdetermination of American forms as *E. pristis* became even greater in the case of Linton's presumed variety of that species, *E. pristis tenuicornis.* Detailed information regarding the morphological features of *R. pristis,* which was not available at the time when Linton was studying the acanthocephalan fauna of New England, render it obvious that *E. pristis tenuicornis* is fundamentally different from *R. pristis* and cannot be regarded as a variety of this species. The presumed variety likewise has no direct relationship with *Nipporhynchus ornatus* based upon Linton's faulty concept of *R. pristis.* It stands as a distinct specific entity which cannot be included within either the genus *Rhadinorhynchus* or the genus *Nipporhynchus.*

The confusion which has resulted from the assumption by early American workers that their material might be identical with species recorded for Europe is no stronger indictment of American than of European investigators. European ACANTHOCEPHALA, which were the first to be named, were usually described briefly and inadequately. Often the original description of a species included mention of only those characteristics which were available for distinguishing the species in question from other European species. Very commonly these conspicuous features were found to be held in common with other species from different parts of the earth. Characteristics which were thus deemed sufficient for specific recognition later became recognized as of generic value. Just so long as the investigators were concerned wholly or largely with the European fauna, the inadequate descriptions offered no

Received for publication, March 4, 1947.

obstacle to the European students, but they misled students on new continents who believed that the local specimens which they encountered were identical with European species because every point in the description of the latter was in complete harmony with the material from another continent. Among the ACANTHOCEPHALA there are still many European species for which adequate information concerning specific details is lacking. Frequently interruptions of exchange of literature and of specimens imposed by war conditions have seriously impeded the integration of taxonomic studies on a worldwide basis.

Since 1918 it has been recognized that *"Echinorhynchus pristis tenuicornis"* is specifically distinct from *Rhadinorhynchus pristis*. The original material on which Linton founded this variety has not been available for reexamination. The present writer has studied a number of series of specimens which seem to agree with the description of *E. pristis tenuicornis*. The first of these collections which came to his attention was a group of individuals collected by Albert Hassall in 1891, subsequently deposited in the Hassall Collection of the United States National Museum under accession number 6324. According to the label accompanying this material, the worms were taken from the intestine of a "trout" at Baltimore, Maryland. It now seems reasonably certain that this host was *Cynoscion regalis* (Bloch and Schneider) commonly called the sea trout, or salt-water trout. This specific identification of the host seems justified since Linton in a later paper (1905) recorded finding *"Echinorhynchus pristis"* in *Cynoscion regalis* at Beaufort, North Carolina.

On the basis of the series of specimens collected by Hassall as supplementing the original characterization by Linton (1892), the present writer (Van Cleave, 1918) redescribed the form which Linton had called *E. pristis tenuicornis*, recognizing it as a distinct species and assigning to it the name *Rhadinorhynchus tenuicornis*. Because of unfamiliarity with procedure in zoological nomenclature, this was regarded as a "new species." Subsequently, other investigators (Meyer, 1932; Chandler, 1934) have accepted this error in judgment and have very generally attributed this species to Van Cleave, 1918. However, since Linton (1892) had applied the name to the subspecies or variety, elevation of his concept to full specific status dictates that Linton still be recognized as the authority for the name at its new taxonomic level. The fact that he had erroneously considered the specimens as a mere variant of *R. pristis* does not impair the availability and validity of the name which he chose for the concept.

In his revision of the genus *Rhadinorhynchus*, Chandler (1934) did not question the generic assignment of *Rhadinorhynchus tenuicornis*. Likewise, in their reconsideration of the family RHADINORHYNCHIDAE, Van Cleave and Lincicome (1940) raised no question as to the propriety of assigning *tenuicornis* to the genus *Rhadinorhynchus*. To the present time no reference has been encountered in the literature regarding possible invalidity of this generic assignment.

Very recently the writer has reexamined all of the specimens in his private collection which seem to approximate the specific description of *R. tenuicornis*. Many of the specimens (Figs. 2, 4) had the praesoma (proboscis and neck) as well as the front part of the trunk so greatly introverted and retracted that specific features like number and arrangement of the proboscis hooks and size and distribution of the body spines were not immediately available for study. Fragmentary bits of evidence on these structures were obtained by examining the introverted parts under

high illumination (Fig. 4). This study has brought out significant facts that have been previously overlooked by some investigators and ignored by others. The most important of these is the observation that at least in some individuals the genital extremity bears a few cuticular spines. This fact alone would rule the specimens out of the genus *Rhadinorhynchus* and suggest that they belong to the genus *Telosentis*. The genital spines (Figs. 10, 11) have the same anomalous orientation as that figured by the present writer (Van Cleave, 1923, figs. b, d) in the description of *Telosentis molini*. Instead of the spines at the genital extremity pointing posteriorly, as they do in all other genera of ACANTHOCEPHALA, at least some of them are obliquely directed toward the anterior end. Very commonly two to eight genital spines can be seen on stained whole mounts and these seem to be limited to the males. In many instances the free ends of the genital spines, especially those farthest from the genital opening, are very much rounded and the internal ends of the spines are poorly delimited. Such spines are doubtless like the ones which Linton observed and termed "papillae" in his discussions on *"Echinorhynchus pristis tenuicornis,"* as narrated below. It should be recalled that the small number of genital spines, limited to males, is in contrast with conditions in *Telosentis molini* and *T. exiguus* in which numerous genital spines are characteristic of both sexes.

In the course of this study, the writer has examined specimens which seem to agree with the original description of *"Echinorhynchus pristis tenuicornis* Linton" from three different genera of marine fishes of wide distribution along the Atlantic and Gulf coasts of North America. These include the following: (a) "Trout" (probably *Cynoscion regalis*) from Baltimore, Maryland, collected by Dr. Albert Hassall; (b) *Micropogon undulatus* from the mouth of the Patunxet River, in Maryland, collected by Dr. Marvin C. Meyer; (c) *Micropogon undulatus* from Galveston Bay, Texas, collected by Dr. Asa C. Chandler; and (d) *Lerimus fasciatus,* taken near Beaufort, North Carolina, by Dr. Wm. E. DeTurk. Differences which at first seemed significant, suggesting the presence of at least two distinct species in this series of collections, were finally explained on the basis of relative degree of obscuring certain features of the proboscis and the extent of the spination of the front part of the body. Following the satisfactory explanation of these apparent differences, it became clear that a single species, *Telosentis tenuicornis* (Linton, 1892), is involved in this series of collections.

As early as 1892 (page 532), Linton may have noticed the genital spines of *tenuicornis,* although he failed to interpret them as spines. The following quotation immediately follows his description of the cuticular spines on the anterior part of the trunk: "The genital aperture of No. 1 [the specimen from *Tylosurus*] as in those typical of the species, is a short distance from the posterior end, and appears to be stellate." In most of the cleared specimens which have been examined in this study the genital spines are so minute and in such small number that they could not impart a stellate appearance such as Linton observed. It is probable that he made his observations on living or alcoholic specimens under reflected light which would make the spines show more clearly.

In 1905, Linton observed and figured genital spines on what he, in his explanation of plates, recorded as *"Echinorhynchus sp.* from gray trout (*Cynoscion regalis*)." However, in the text of the same article (page 384), in listing the parasites of *Cynoscion regalis,* he seemed to consider these same worms as *"Echinorhynchus*

pristis Rudolphi" since under this specific heading he wrote "There was also found in this lot the posterior end of a female of this genus which was provided with papillae (Figs. 15, 16)." The papillae to which he referred are obviously rather blunt cuticular spines such as the present writer has observed in other specimens which had been tentatively identified as *R. tenuicornis*. Since the specimen on which Linton observed this genital armature was but a fragment, it is highly probable that he was in error as to the sex, since spines seem to be limited to males.

It is an extremely peculiar coincidence that the same year (1905) in which Linton published observations on the occurrence of cuticular spines on the genital extremity of *"Echinorhynchus pristis"* (= *Telosentis tenuicornis*). Porta misconstrued the identity of specimens mentioned in another record by Linton to further complicate interpretation of Linton's observations on the ACANTHOCEPHALA of marine fishes of New England. In the same paper in which Linton gave the original description of *"Echinorhynchus pristis tenuicornis"* (1892), he recorded the finding of encysted post-larval acanthocephalans in the peritoneum of *Lophius piscatorius, Paralichthys dentatus,* and *Pomatomus saltatrix*. These juvenile worms he tentatively identified as *"Echinorhynchus incrassatus* Molin" although he pointed out certain points wherein they failed to agree with Molin's description. In his detailed description of these specimens, Linton (1892: 533) remarked that the "rounded extremity is armed with very small sagitate spines." The illustration accompanying his description of *"E. incrassatus"* are unquestionably drawings of immature specimens of an undetermined species of the genus *Corynosoma* as previously suggested by Van Cleave (1920: 167). Without proper evaluation of the body shape, the form and armature of the proboscis and nature and distribution of the body spines, Porta (1905) assumed on the basis of genital spines alone that these worms were identical with specimens which he had identified as *"Echinorhynchus lateralis* Molin" (= *Telosentis molini* Van Cleave) from European fishes.

Although *Corynosoma* and *Telosentis* belong to entirely different families (POLYMORPHIDAE and RHADINORHYNCHIDAE) they have several distinctive structural features in common, even though these features in themselves show distinctive differences in the two genera. As previously mentioned, the presence of body spines, some of which are distributed around the genital extremity, is one point of similarity although the cuticular spines are considerably different in form and arrangement in the two genera. Another point in common is the high degree of development of the specialized musculature for introversion of the body at both anterior and posterior extremities. In many individuals of *Telosentis tenuicornis,* the musculature for introversion of the posterior extremity (Fig. 9) is extremely well developed. For the genus *Corynosoma* this specialized condition of the posterior extremity of the trunk to form a genital vestibule was emphasized (Van Cleave, 1945) in a special article dealing with its morphological and taxonomic significance. At that time, similar conditions in the family RHADINORHYNCHIDAE had not been observed. In fact, the specialized "vestibular muscles," which the writer (Van Cleave, 1914: 277) mistakenly designated as the "fan ligament," are so strongly developed in *T. tenuicornis,* that by their contraction during histological fixation the entire genital tract is often torn loose from the body wall (see Fig. 8). In similar manner, the retractors colli at the anterior end of the body are so violently retracted in many preserved specimens that along with the complete introversion of the proboscis and

retraction of the receptacle and neck, the inner layer of the body wall is torn loose (Fig. 4), presenting a most anomalous condition of distortion. Under these conditions, the internal organs become completely dissevered from their connection with the outer body wall and the part which appears to be the surface of the animal is only the cuticula and a very small part of the adjacent subcuticula. The remainder of the subcuticula and the body musculature invest the retracted internal organs which have been entirely detached from the body covering.

Chandler (1934: 354) expanded and emended the specific description of *T. tenuicornis*. As a very minor point, he failed to observe the genital spines but that oversight is not surprising since most of his specimens were females and in the male which he figured the posterior extremity is twisted so that a full lateral view, best suited for viewing the genital spines, was not available. In describing the crescent of large hooks on the ventral surface of the base of the proboscis (Figs. 1, 2, 4, 5, 6), he cited lengths of 40 to 50 μ. In some of the individuals observed by the present writer the hooks of this crescent attain 79 μ. Similarly, the spines on the fore part of the trunk are often longer than the 60 to 75 μ cited by Chandler, since some individuals from *Lerimus* had body spines up to 87 μ. In the statement "Proximal fourth of proboscis bare" Chandler has mistaken the neck (Figs. 1, 3) for a portion of the proboscis. Rauther (1930) was the first student of the ACANTHOCEPHALA to clearly differentiate between the proboscis and neck. He proposed the term praesoma for the entire hold-fast organ anterior to the cuticular infolding at the anterior extremity of the trunk. Many writers have used the term proboscis as the exact equivalent of the entire praesoma, apparently restricting the term neck to any conspicuously modified region between the thorn-covered proboscis and the trunk such as is characteristic of *Pomphorhynchus* and several other genera.

Many investigators have directed especial attention to the large, inordinately branched nuclei that occur in the subcuticula of some species of ACANTHOCEPHALA (Van Cleave, 1928). In some individuals of *T. tenuicornis*, fantastically branched nuclei practically encircle the entire body, as shown in Fig. 7. These could not be seen in some specimens, but the individual from which the figure was drawn was a fully gravid female, hence the condition could not be a transitory phase preparatory to the amitotic fragmentation of the subcuticular nuclei (Van Cleave, 1928: 112). Meyer (1932) mentioned the presence of large branching nuclei as characteristic of the genus *Leptorhynchoides* which belongs to the same family as *Telosentis*.

The assignment of *Rhadinorhynchus tenuicornis* to the genus *Telosentis* brings to three the number of species attributable to this genus. Originally founded on a single species, *T. molini* from *Atherina hepsetus* from the Mediterranean, the distribution of *Telosentis* seemed limited to southern Europe (Meyer, 1932), especially after Kostylew (1926) added a second species *T. exiguus* (von Linstow, 1901) from *Engraulis encrasicholus* of the Black Sea and showed that *T. exiguus* likewise occurs in *Atherina pontica*, also of the Black Sea. The present article is the first to record the presence of a species of *Telosentis* outside southern Europe.

Along the Atlantic and Gulf coast of North America from Woods Hole, Massachusetts to Galveston Bay, Texas, *T. tenuicornis* has been recorded from at least eight genera of marine fishes. The following serve as definitive host for *Telosentis tenuicornis: Cynoscion regalis, Micropogon undulatus, Lerimus fasciatus, Tylosurus acus, Lobotes surinamensis, Paralinurichthys perciformis, Leiostomus xanthurus*, and

Polynemus octonemus. The last two of these were added in a note on distribution of this parasite by Chandler (1935). In the same note Chandler called attention to localization of infections by *T. tenuicornis* within the species of fish which serve as its definitive hosts in Galveston Bay. It is highly probable that suitable intermediate hosts are lacking in the regions where he found normal definitive hosts free of parasites. In compiling the above host list, the writer was constantly aware of the fact that many of the records by Linton (1892, 1901) made no distinction between his *"Echinorhynchus pristis"* (*Nipporhynchus ornatus*) and the form which he considered as a variety under the name of *"Echinorhynchus pristis tenuicornis"* (*Telosentis tenuicornis*).

Because of the wide geographical distribution of the genus and peculiar distribution of its species it seems desirable to present a key for the differentiation of the species of *Telosentis*.

KEY TO THE SPECIES OF THE GENUS TELOSENTIS

1 (a). Proboscis clavate, considerably more swollen near tip than at base. All hooks at base of proboscis, small, of about uniform size. Anterior trunk spines small, less than 50 microns 2

(b). Proboscis cylindrical. A crescent of about eight hooks on ventral surface at base of proboscis conspicuously enlarged. Anterior trunk spines long (at least 70 microns) ..... *Telosentis tenuicornis* (Linton, 1892)

2 (a). Many of proboscis hooks protruding from a cuticular elevation or theca. *Telosentis exiguus* (von Linstow, 1901)

(b). Proboscis hooks lacking cuticular theca around base. *Telosentis molini* Van Cleave, 1923

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EXPLANATION OF PLATE

Telosentis tenuicornis (Linton, 1892)

Details of structure, from stained permanent mounts in damar or clarite, drawn by Katherine Hill Paul. The scale accompanying Fig. 1 has the value of 0.1 mm and applies to all figures on this plate except 5 and 6 for which the scale with Fig. 6 has the value of 0.05 mm.

ABBREVIATIONS

G.P.—genital pore
G.S.—genital spines
G.V.—genital vestibule
N.—boundary between neck and trunk
V.C.—ventral crescent or proboscis hooks
V.M.—vestibular muscle

FIG. 1. Portion of the proboscis, the neck and portion of the trunk of a female individual from *Micropogon undulatus,* showing how a small part of the anterior end of the trunk may be retracted and invaginated without obscuring the neck.

FIG. 2. Anterior end of a female from *Micropogon undulatus,* showing proboscis fully extended but entire neck and a portion of the anterior extremity of the trunk retracted within the body cavity, obscuring many of the largest cuticular body spines.

FIG. 3. Anterior region of trunk of a male, fully extended, showing arrangement and size differentiation of body spines. Specimen from *Lerimus fasciatus.*

FIG. 4. Anterior region of a female from *Cynoscion sp.,* showing extreme invagination of proboscis, neck, and trunk. Introversion at time of fixation was so violent that the cuticula with its hooks and spines was stripped free from part of the proboscis, neck, and trunk.

FIG. 5. Crescent of large hooks at base of proboscis of a female, as seen in lateral view. Broken lines indicate hooks on far side of proboscis.

FIG. 6. Crescent of large hooks at base of proboscis of a male, as seen in anterior view at accidental kink in proboscis.

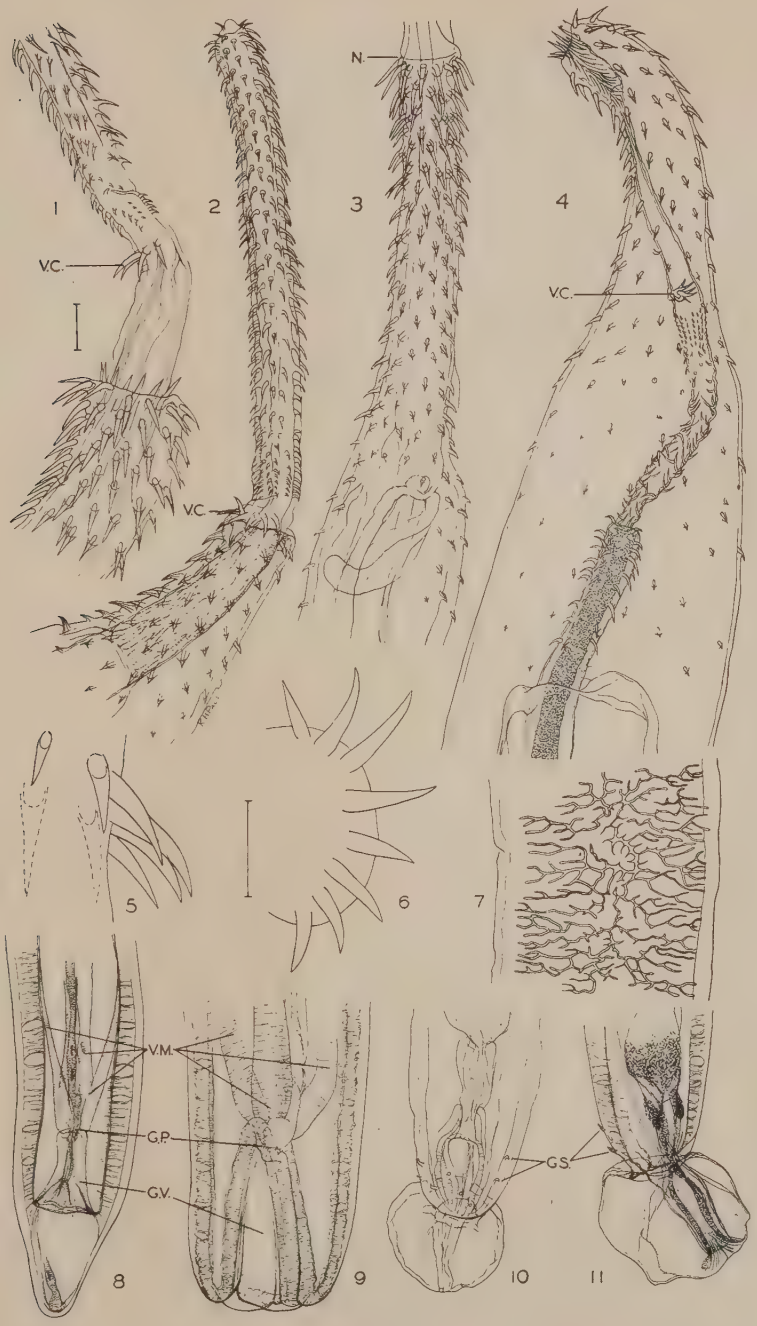
FIG. 7. A portion of the body wall of a gravid female, showing portion of an extremely branched subcuticular nucleus extending the entire width of the body.

FIG. 8. Posterior extremity of a female in which violent contraction of the vestibular muscles (V.M.) pulled the genital vestibule (G.V.) free from the trunk wall.

FIG. 9. Posterior extremity of a female showing genital vestibule (G.V.) and vestibular muscles (V.M.) in normal state.

FIG. 10. Dorsal view of posterior extremity of a male with bursa extruded, showing genital spines (G.S.) and their arrangement.

FIG. 11. Lateral view of posterior extremity of a male with bursa extruded, showing peculiar orientation of genital spines (G.S.).



PLATE

THE EFFECTS OF COLD TEMPERATURES ON THE EGGS OF *SCHISTOSOMA JAPONICUM*

ERNEST CARROLL FAUST*

INTRODUCTION

The known endemic regions of schistosomiasis japonica include five relatively small areas in the Japanese home islands, a vast territory in the Yangtze valley of Central China and several watersheds in South China, a small focus in Formosa, portions of the islands of Mindoro, Leyte, Samar, and Mindinao in the Philippines, and one small area in northern Celebes. In Japan and along the south coast of China the molluscan intermediate host is *Oncomelania* (*Katayama*) *nosophora;* in the Yangtze valley it is *O. hupensis;* in Formosa, *O. formosana,* and in the Philippines, *O. quadrasi.* The intermediate host in Celebes has not been identified.

The midsummer temperatures in all of these countries is quite warm and during this season conditions are favorable for the hatching of mature viable *Schistosoma japonicum* eggs that are deposited in, or are carried into the backwaters of streams in all endemic foci. Temperatures satisfactory for hatching of the eggs prevail during the other seasons of the year in Celebes, the Philippines, and Formosa. In South China the midwinter temperature is cool, with occasional frost, while in Central China and in the upper tributaries of the Yangtze river in West China the winters are moderately cold, with periods of ice and snow. In the endemic areas of Japan the winter period is considerably more prolonged and more severe.

As the fall season comes on in China and Japan the hatching of mature viable eggs slows down and then ceases. A question of both theoretical and practical interests is this: will the eggs whose hatching is inhibited by cold weather survive in a viable state and hatch the following spring? Opportunity to study this problem was provided when three infected dogs were shipped from Leyte, P. I., to the National Institute of Health, Bethesda, Maryland, and stool specimens from these animals were made available for examination.

Grateful acknowledgment is made to Preventive Medicine Service, Office of the Surgeon General, U. S. A., Washington, D. C., and to Doctors Willard H. Wright, Fred J. Brady, Eloise B. Cram and Myra Jones, of the National Institute of Health, for the opportunity afforded. Thanks are also extended to Mrs. Frances Willard Fuller for technical assistance.

MATERIAL AND TECHNIC

Two of the host animals had natural infections of many months duration, as indicated by the presence of numerous calcified eggs in their stools. The third animal had been experimentally exposed to 5,000 cercariae by T/Sergeant Preston M. Bauman ten weeks previous to its arrival in the United States. A sedimented specimen of stool from the first dog (specimen 1) was forwarded by air express from Bethesda, and sedimented and mucous samples of stool from the second dog (specimens 2 and 3) were shipped from an Ohio river city to the writer in New Orleans.

Received for publication, December 28, 1946.

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The third dog, which had been expressed to New Orleans for study, was passing dysenteric stools containing many hookworm and a few *S. japonicum* eggs.

On arrival in New Orleans the sedimented samples showed extensive bacterial growth and were repeatedly centrifugalized in pure spring water at 1500 rpm until the supernatant liquid was clear. The mucus specimen appeared to be relatively free of bacteria and no attempt was made to separate nests of eggs from their mucous menstruum. Specimen 1 was received on September 8, 1945, and specimens 2 and 3 on October 31, 1945. After preliminary sampling each specimen was placed in a 13×100 ml test tube and stored in an electric ice box at a temperature of $4° \pm 1°$ C. The dysenteric stools of the experimentally infected dog were used primarily for another investigation (Faust, Ingalls and See, 1946) and were employed in the present study only to compare the quality of the refrigerated eggs with those freshly passed by this animal.

At times of examination the specimens were removed from the ice box and approximately 0.2 ml of the sediment (or mucus) was transferred from the test tube to a 37×75 mm slide, then mounted with two 22-mm square coverslips. However the preparations made on February 14 and March 6 consisted of 0.4 ml of material placed on two slides with double coverslip mounts. Each preparation was allowed to reach room temperature ($25° \pm 4°$ C), after which all eggs in the mounts were examined to determine their stage of development, viability, or degeneration.

In a recent study of *Schistosoma japonicum* eggs obtained from human and canine sources on Leyte the writer (Faust, 1946) has indicated the usefulness of grouping these eggs in the following categories: immature viable, mature viable, degenerate, calcified, and shells. Immature viable eggs were distinguished on the basis of a small-sized shell, at times with an abbreviated spine, and a larva which lacked epithelial cilia and had incomplete or little internal differentiation. The cellular organization and the appearance of the cytoplasm were normal for viable larvae. Eggs were designated as "mature viable" when they had epithelial cilia and beating "flame cells." Almost invariably the cilia began to move energetically as soon as the miracidia were warmed to room temperature, at which time the larvae usually began to move around inside their shells. Frequently, hatching of mature viable eggs occurred while the preparation was being studied. Degenerate eggs were either immature or mature ones in which granulation or vacuolization of the cytoplasm had taken place and in which the epithelial cilia and embryonic membrane, if previously developed, had broken down. Calcified eggs are also degenerate ones, but partial or complete calcification indicated that the larva had died before evacuation from the host's tissues. Thus, it constituted a convenient index of host's reaction to the presence of the egg after escape from a blood vessel into perivascular tissues. Empty shells indicated that larvae had escaped either normally in the act of hatching or consequent to degeneration.

PRESENTATION OF DATA

The essential data are summarized in Table 1. It will be observed from examination of the table that the number of eggs picked up in 0.2 ml of sediment (or mucus) varied from 32 to more than 100. Although this variation may have some slight bearing on the relative significance of the several observations, it does not alter certain trends which are deduced from the data. In the first place, there was a fair proportion of degenerate and calcified eggs in the specimens at the time they were

received; yet in the sedimented samples more than half (specimen 1, 57%; specimen 2, 55%) were viable at this time. Moreover, several free-swimming miracidia in the early samplings suggested that a fair proportion of the shells represented viable eggs. As the observations progressed the relative proportion of viable and degenerate eggs in the sedimented samples was reversed. The immature viable eggs showed this trend earlier than the mature ones; this was expected since they probably contributed to both the mature and degenerate forms.

Even in the absence of gross bacterial contamination the degeneration of eggs enmeshed in masses of mucus occurred much more rapidly than it did for the sedimented eggs, and no viable ones were found 68 days after the first examination. Yet it is possible that if 50 or more per cent rather than 15.8 per cent of the mucus-trapped eggs had been viable at the beginning of the study, there might not have been this discrepancy between the eggs in the two types of material.

TABLE 1.—*Data on eggs of* Schistosoma japonicum *in stool specimens kept at refrigerator temperature and examined periodically to determine viability*

Date	Stool specimen	No. eggs examined	% immature viable	% matture viable	% degenerate	% calcified	% shells
Sept. 8, '45	1	100	20.0	37.0	11.0	8.0	24.0
Oct. 20	1	56	14.3	51.8	12.5	7.1	14.3
Oct. 31	1	74	9.5	35.1	39.2	10.8	5.4
	2	60	21.7	33.3	21.7	13.3	10.0
	3	47	6.4	9.4	55.3	12.9	14.9
Nov. 27	1	65	9.2	27.7	32.3	4.6	26.2
	2	36	11.1	33.3	27.8	16.7	11.1
	3	32	3.1	9.4	81.3	...	6.2
Jan. 7, '46	1	102	2.0	13.7	77.4	6.9	...
	2	108	4.6	8.3	71.3	4.6	11.1
	3	86	...	...	95.3	4.7	...
Feb. 14	1	125	1.6	5.6	77.6	2.4	12.8
	2	93	2.1	4.2	74.5	16.8	...
	3	199	...	...	92.0	2.0	7.0
March 6	1	99	1.0	1.0	82.0	7.0	9.0
	2	148	...	2.0	74.3	4.7	18.9
	3	175	...	...	77.7	...	22.3

A small proportion of the eggs in specimen 1 remained viable at approximately 4° C for a period of six months and about the same proportion of eggs in specimen 2 remained viable for a period of four months, at which time the experiment was terminated.

DISCUSSION

The cold-temperature tests carried out in this study on the eggs of *Schistosoma japonicum* parallel one test of Faust and Meleney (1924, p. 20, Ser. A, Exp. 2) except that in the earlier experiment examination to determine viability was made after only 20 hours at 4° C. In specimen 1 of the present series a high percentage of the originally viable eggs retained their viability from September 8, 1945, through November 27, but by January 7, 1946, about 50 per cent of the previously viable eggs had degenerated. A somewhat more rapid degeneration occurred in specimen 2 from October 31 through January 7. An additional 50 per cent loss in viability in each of these two samples was sustained during each of the two following months.

The maximum period of high percentage viability which was observed (specimen 1) amounted to 80 days, although a few eggs survived for more than twice that period. From a practical viewpoint the duration of high viability at a temperature considerably lower than the minimum for hatching corresponds to the cool season in

South China but is somewhat less than that in Central China and only about half that of endemic areas of schistosomiasis in Japan. This suggests that viable eggs which reach fresh water in South China after the temperature is too cool for hatching stand a good chance of wintering over and of producing a good "hatch" on the advent of spring. In Central China a smaller "hatch" may be expected because of the longer winter season, and in Japan the prolonged winter would usually exceed the viable state of the majority of the eggs.

It may be objected that the source of material utilized was from a region where the temperature is favorable for the hatching of the eggs of *S. japonicum* during the entire year, whereas the suggested practical implications are for endemic areas with a cool or cold winter season. If any racial or strain differences in *S. japonicum* should ever be demonstrated as an adaptation to climate or to different species of molluscan hosts, there remains the evidence that eggs of this blood fluke from a tropical endemic area have remained viable for an appreciable period of weeks at a temperature of 4° C and that a high percentage of individuals has retained the ability to hatch and swim about in the water similar to eggs recently evacuated from their host, or those previously studied by Faust and Meleney (1924) from sources in Central China and Japan.

SUMMARY

The effect of continued cold temperature (4° ± 1° C) has been tested on eggs of *Schistosoma japonicum* passed in stools of two heavily infected dogs shipped from Leyte, P. I., to the United States. One sedimented specimen of egg-containing feces was examined periodically from September 8, 1945, through March 6, 1946, while a sedimented specimen and one with eggs trapped in mucus from a second dog were similarly studied from October 31, 1945, through March 6, 1946.

Starting with an initial percentage of viability somewhat above 50 in each of the sedimented specimens, the first maintained a high percentage of viability for 80 days and the second for at least 27 days, after which time there was a decline in the number of hatchable eggs in both samples. Nevertheless, in both samples a few eggs remained viable through March 6, 1946, when the experiment was terminated. The mucus specimen initially had a considerably lower percentage of viable eggs and none were demonstrated after the twenty-seventh day.

Since the period of high viability of these eggs at cold temperatures corresponds to the cold season in South China, it is suggested that in this endemic area a considerable proportion of eggs may survive and hatch in the spring. A smaller percentage of eggs would over-winter in Central China where the cool season is longer, and very few would remain viable at the end of the prolonged, rigorous winter in endemic areas in Japan.

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A METHOD FOR PROVIDING A CONSTANT SUPPLY OF TROPICAL RAT MITES, *LIPONYSSUS BACOTI,* INFECTED WITH THE COTTON RAT FILARIA, *LITOMOSOIDES CARINII**

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Methods for raising and handling tropical rat mites for infection experiments with *Litomosoides carinii* have been placed on a routine basis as a result of experiments extending over the past year. Our experience agrees with that of several other investigators in indicating that this mite is more difficult to raise in the laboratory during the summer than during our mild winter. We will not be certain that we have overcome all of the seasonal difficulties until after the next summer, but we have hopes that we have done so, since our present methods were developed as a response to the difficulties of the past summer.

EQUIPMENT

The galvanized iron tanks in which the mites are raised are a modified form of those used for the mass infection of cotton rats (Scott and Cross, 1946). The present design is shown in Fig. 1. The most convenient size is 12″ × 15″ and 12″ deep. To prevent the mites from escaping, oil is kept in a 1″ × 1″ moat, which is soldered around the outside of the tank about 2″ down from the top. The cover is made of ½″ mesh hardware cloth supported 2″ below the top by heavy wire slings. Beneath a hole in the cover there is fastened a feeding basket 5″ in diameter and 6″ in over-all height. It is constructed of two cylindrical bands of galvanized iron connected by four uprights. The upper band is one inch in height and the lower one and one-fourth. The food container is in the shape of an inverted cone 4″ in height, made of ½″ mesh hardware cloth and soldered into the upper cylindrical band. Over the top of the lower cylindrical band is soldered a floor of 1/3″ mesh hardware cloth. A galvanized iron pan slips over the bottom and fastens with a loop of wire. This pan serves to catch all food crumbs and virtually eliminates difficulties with cheese mites. About 2″ of wood shavings are placed in the bottom of the tank and covered with a sheet of hardware cloth. On this is placed a 3- or 4-inch layer of hay, both shavings and hay having been previously baked in an oven to kill any cheese mites or predacious mites they may contain.

PROCEDURE

It is our impression that the mites can be raised more readily on white rats than on cotton rats, although we have not run enough parallel experiments to prove this assumption and the observations of Bertram et al (1946) would not support it. At any rate, we find it more convenient to start our colony on a white rat on grounds of availability, ease of handling, and color. A variable number of mites is added to start the colony, about 50 being the most satisfactory number, although several times we have started with only 10 or 15. The rats are fed rat pellets and all the carrots they desire, but are given no water.

Received for publication, December 31, 1946.

* This work was supported in part by a grant for the study of filariasis from the John and Mary R. Markle Foundation.

About once a week one person lifts the top of the tank while another removes the pan from the bottom of the feeder, lays it in a deep white enamel basin and attaches a clean pan to the feeder. The food crumbs in the pan are then sifted a few at a time through a 20-mesh wire screen into another white basin. As the mites crawl out of the food they can be picked up with a glass tube attached to a suction bottle.

The number of mites found in the feeder pan is a rough indication of the progress of the colony as is also the number of mites seen crawling on the white rat. An excessive number will be evidenced by large numbers crawling up the sides of the tank, a situation which calls for a transfer of the rat to prevent its being bled to death. Serious damage to the rat has not occurred within 3 weeks in any case, although using the same rat to start colony after colony eventually results in anemia, as well

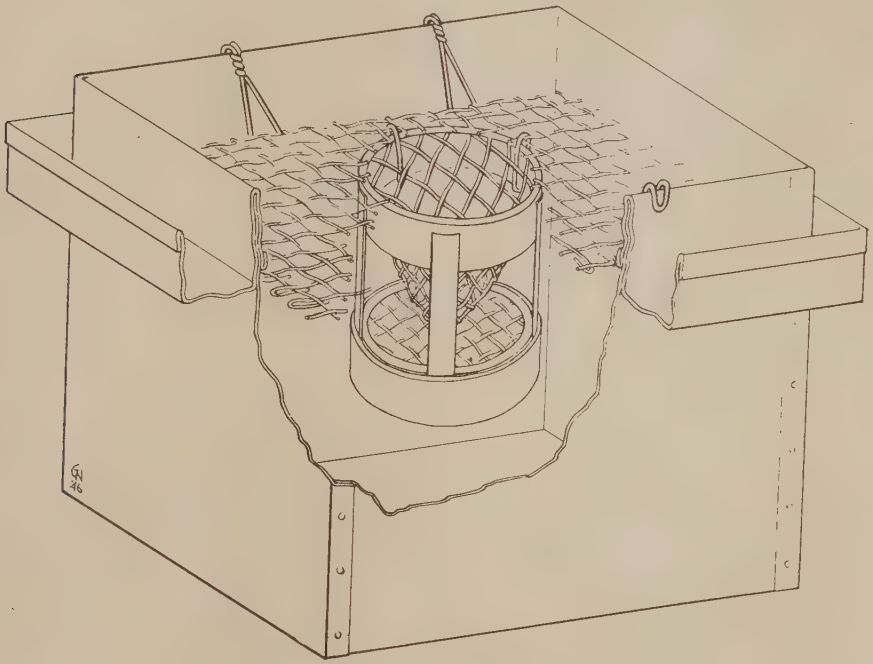

FIG. 1. Tank and feeder for raising tropical rat mites.

as open sores on the back. After being cleared of mites and given a rest for several weeks, the rat can be again used. Since the accumulation of excreta seems to be inimical to the continuance of the mite colony, the white rat is always transferred out of the tank by the end of the fourth week. As a routine, a clean unit is prepared weekly and a white rat transferred into it along with any mites that may be attached. If, however, the number of mites on the rat seems to be large, some of them are brushed back into the tank before transfer. If the number seems to be small, mites taken from the feeding pan are added to start the new colony.

After the white rat is removed a cotton rat infected with *L. carinii* is added to the tank and allowed to stay for about one or two weeks. When removed, it is dropped into an ether jar with a white paper in the bottom. When the rat has just lost its ability to respond to a stimulus, it is removed and brushed vigorously from tail to

head over a white pan. Virtually all of the mites will be removed by this process. They and the ones found on the paper in the ether jar will recover with no apparent damage to the larvae of *L. carinii* they may harbor. As a rule the schedule is arranged so that this cotton rat can go directly into another tank. If not, it is put into a cage and dusted with rotenone and not used again for several weeks. The cover of the tank with the attached feeder is removed and checked for mites at this time.

Within a few days after the cotton rat has been removed from the tank the mites will start crawling up the sides and can be transferred to vials with a suction tube. The emergence can be hastened by piling the hay against the sides of the tank and applying gentle heat to the bottom or directing the beam from an infra-red heating lamp downward into the center of the tank. If complete removal of all mites is required, the best procedure seems to be vigorous shaking of small quantities of the hay in a sieve over a white pan. An apparatus to remove the mites from small quantities of material by the application of heat has been described by Bertram (1946). At present we are attempting to develop a modification of this apparatus to handle material on a large scale. If large numbers of mites are not wanted simultaneously, the tank can be held for two weeks or so after the rat is removed and the mites recovered as they crawl up the sides. The mites are held in rubber-stoppered shell vials and do not usually suffer an excessive mortality during a period of 2 weeks after their last opportunity for a blood meal, while many live much longer.

By having 7 or 8 units available, a schedule can be set up so that all transfers are made on the same day each week, except when a colony shows unusual abundance, a rat becomes ill, or some other irregularity forces a change in schedule. Other than these weekly transfers the daily care consists only in dropping food into the baskets and removing mites from the tops of tanks which have completed the cycle.

DISCUSSION

No tests have been made to determine whether the methods described here are superior to those described by Williams (1946), Olson and Dahms (1946), or Bertram et al (1946) with regard to the number of mites raised per rat. They simply represent methods which in our hands have made possible setting up a routine of care requiring the minimum of planning and relatively little daily attention.

In this method the exact date on which the mites become infected is not established, but the routine of allowing meals of infected blood over a period of ten days does produce many infected mites. Most of these mites will still be alive 2 weeks after the rat has been removed and at this time all of the larvae in them can be expected to have reached the infective stage. As an indication of the extent of infection in mites raised and infected in this manner the results of two series may be mentioned. Each mite was placed in a drop of saline under a cover glass and examined with a compound microscope using 10 × eyepieces and an 8-mm objective. If no larvae were seen moving within a period of ½ minute or so, they were declared negative. Some positives were undoubtedly missed in this cursory examination which was made for the purpose of obtaining a number of positive mites in a short length of time. In these two series 24 per cent of 180 mites and 20 per cent of 209 mites respectively were found to be infected.

SUMMARY

Routine methods have been developed to provide, with a minimum of planning and daily care, a constant supply of large numbers of tropical rat mites, *Liponyssus*

bacoti, infected with the cotton rat filaria, *Litomosoides carinii.* The mites are raised on a white rat in a galvanized iron tank surrounded by an oil moat. A feeder with removable crumb pan virtually eliminated the nuisance of cheese mites and permits weekly assessment of the progress of the colony. After 3 or 4 weeks an infected cotton rat is substituted for the white rat. Ten days later this rat is removed and in a few days the hungry mites begin to crawl up the sides of the tank and can be removed to bottles by suction. Approximately one-fourth of the mites so raised are infected.

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RELATIVE EFFICIENCY OF SIX SPECIES OF MOSQUITOES FROM GUAM, M. I., AS DEVELOPMENTAL HOSTS FOR *DIROFILARIA IMMITIS*

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Dog heartworm, *Dirofilaria immitis* (Leidy), has long been an important and difficult problem confronting owners of dog kennels and individuals who keep dogs for pets. The wide use of dogs in World War II has greatly increased the economic importance of this disease, as they are an expensive, but vital part of certain combat operations. A routine survey on Guam, M. I., demonstrated the degree to which dogs employed in warfare were exposed to *Dirofilaria* infections. Eighteen of 20 local dogs were found to be infected with heartworm. In ten of these the microfilaria counts ranged from 11,500 to 41,900 per milliliter of blood; in the other eight, the counts ranged from less than 100 to 7,500 per milliliter. The technique for making these counts was that described by Franks, Chenoweth, and Stoll (1947). Data are presented here to show which species of mosquitoes are most likely to be transmitters of dog heartworm on Guam.

MATERIALS AND METHODS

Studies were made on six of the nine known species of mosquitoes on Guam (Nov. '45 to Jan. '46): *Culex sitiens* Wied., *C. annulirostris* Skuse, *C. quinquefasciatus* Say, *Aedes aegypti* (Linn.), *A. pandani* Stone, and *A. guamensis* Farner and Bohart. The adult mosquitoes were reared in the laboratory either from colonies or from larvae that were collected in the field. After allowing these mosquitoes to feed on an infected dog, they were kept in small cages for the microfilariae to develop. A 10 per cent sugar solution was provided as food for the caged mosquitoes. Dissections were made at intervals during the developmental period to determine the fate of the ingested larvae.

Three species, *C. quinquefasciatus, C. annulirostris,* and *A. guamensis* did not feed readily on the dog, whereas *C. sitiens, A. aegypti,* and *A. pandani* did feed readily. With the exception of *C. quinquefasciatus,* the mosquitoes were fed on the dog during the hours between 1900 and 2200. To obtain an adequate number of engorged *C. quinquefasciatus,* the dog was exposed during the entire night to this species.

It was necessary to remove the *A. guamensis* and *A. pandani* before they were completely engorged because, if they were allowed to engorge, all mosquitoes died within 36 hours, and over 90 per cent died within 12 hours. Apparently the larvae caused sufficient injury to the digestive systems to kill the mosquitoes, although no larvae were seen that had penetrated the digestive tube within this period and no injury was noted on microscopic examination. The mosquitoes exhibited nervous tremors prior to death. Shortly after death the entire digestive tube, including the

Received for publication, February 7, 1947.

[1] The writer wishes to express his appreciation for the assistance given by J. F. Spitzer, PhM2c, L. L. Darrow, PhM3c, and J. H. Stewart, PhM3c, on this problem, and for the data on the incidence of *Dirofilaria* on Guam, supplied by Lt. Comdr. M. B. Franks, MC(S), USNR, and Lt. Comdr. N. R. Stoll, H(S), USNR.

Malpighian tubules, were in process of disintegration, liberating the stomach contents into the body cavity. It is likely that these two species of mosquitoes do not survive under natural conditions to develop infective-stage larvae after feeding on a heavily infected dog. The microfilaria counts in the dog varied from 43,400 to 47,750 per milliliter of blood during the feeding period.

RESULTS

The data in Table 1 show that *Culex* ingest many more microfilariae with a single blood meal than do *Aedes*. The greatest number of microfilariae taken in a single blood meal by a *Culex* was in the case of *C. quinquefasciatus,* with a total of 604; in *Aedes* the greatest number, in individuals that were allowed to engorge completely, was in the case of *A. aegypti,* with a total of 54. This difference is due in part to the larger stomach capacity of *Culex*. During the first 2 days after feeding, two additional differences were noted between the *Culex* and the *Aedes:* (1) the microfilariae migrated from the stomach to the Malpighian tubules in greater numbers in *Aedes* than in *Culex,* and (2) nearly all microfilariae larvae that remained in the stomach of *Culex* on the second day were dead, whereas a high percentage was still alive in the *Aedes*.

TABLE 1.—*Average number and location of developing* Dirofilaria immitis (*Leidy*) *larvae in six species of mosquitoes. Guam, M. I., 1945–1946*

Species of mosquito	Microfilariae larvae*			Sausage-shaped larvae†		Infective larvae‡	
	§	Location of larvae		§	Location of larvae	§	Location of larvae
		Stomach	Mal. tubules		Malpighian tubules		Body cavity
Aedes guamensis	25	19.3	30.0	25	8.1	25	5.5
Aedes pandani	25	11.4	21.4	25	4.7	37	2.3
Culex annulirostris	25	80.6	0.4	25	0.9	100	0.6
Culex quinquefasciatus .	25	172.8	1.5	25	0.7	120	0.4
Aedes aegypti	25	21.3	17.0	29	1.6	86	0.2
Culex sitiens	25	122.2	4.3	136	0.3	86	0.2

* 1–2 days after being ingested.
† 3–11 days after being ingested.
‡ 12–16 days after being ingested.
§ Number of mosquitoes.

Counts made during the period of development of the larvae in the Malpighian tubules showed that the *Culex* lost nearly all of them before they reached the sausage-shaped stage. The slightly higher number of sausage-shaped larvae in the case of *C. annulirostris* was probably due to the error in sampling. The *Aedes* all retained greater numbers of larvae in the Malpighian tubules than did the *Culex*. The greatest number of sausage-shaped larvae in *Culex* was in *C. quinquefasciatus*, with a total of 10; in *Aedes,* the greatest number was in *A. guamensis,* with a total of 32.

The infection rate in all six mosquitoes was low for the infective-stage larvae. The largest number of such larvae in a single *Culex* was in *C. sitiens,* with a total of 10, five of which were in the labium; in *Aedes,* the greatest number was in *A. guamensis,* with a total of 25, nine of which were in the labium. The total of infective-stage larvae was largest in *A. guamensis* and *A. pandani,* with averages of 5.5 and 2.3, respectively. Rosario (1936) found *C. fatigans* = *C. quinquefasciatus* very susceptible to infection. Summers (1943) regarded this species as having little importance as a transmitter in southern Louisiana. It was interesting to note that in *A. aegypti* large numbers of larvae migrated to the Malpighian tubules from the stomach, and

that in this species there was a much larger drop in the numbers that reached the sausage stage than in the other two species of *Aedes*. Only two mosquitoes of *A. aegypti*, out of 86, contained infective-stage larvae; one contained 16, all in the labium, and the other contained only two, which were both in the labium and were dead.

The data in Table 2 show that only 2 per cent of *A. aegypti* contained infective-stage larvae and this was the least efficient developmental host of the six species of mosquitoes. Summers (1943) reported *A. aegypti* as being infected only to the sausage stage. Rosario (1936) reported this species to be quite susceptible to

TABLE 2.—*Percentages of mosquitoes infected with the various stages of* Dirofilaria immitis (*Leidy*), *showing the locations of the larvae in the hosts. Guam, M. I., 1945–1946*

Days after feeding	Mosquitoes		Per cent mosquitoes with larvae in various body locations				
	Total no.	Per cent with larvae	Stomach	Malpighian tubules	Labium	Head capsule	Body cavity*
Culex sitiens							
1–2	25	100	100	36	0	0	0
3–11	136	19	0	18	1†	1†	1†
12–16	86	15	0	0	14	4	5
Culex annulirostris							
1–2	25	100	100	28	0	0	0
3–11	25	44	0	44	0	0	0
12–16	100	27	0	0	20	7	6
Culex quinquefasciatus							
1–2	25	100	100	48	0	0	0
3–11	25	24	0	24	0	0	0
12–16	120	23	0	0	11	5	16
Aedes aegypti							
1–2	25	100	100	100	0	0	0
3–11	29	38	0	38	0	0	0
12–16	86	2	0	0	2	0	0
Aedes pandani							
1–2	25	100	100	100	0	0	0
3–11	25	80	0	80	0	0	0
12–16	37	57	0	3‡	49	24	22
Aedes guamensis							
1–2	25	100	100	100	0	0	0
3–11	25	100	0	100	0	0	0
12–16	25	68	0	0	60	36	60

* Includes thoracic, abdominal, or leg cavities.
† Infective larvae (11th day).
‡ Degenerating sausage-shaped larvae (12th day).

infection. In *A. guamensis* and *A. pandani* 68 and 57 per cent, respectively, were infected, showing that these two species are the most efficient.

A detailed study was not made of the developmental cycle in the mosquitoes. Generally, the filariform larvae changed to sausage-shaped forms between the second and third days, and from sausage-shaped to infective stages, which were free in the body cavities of the mosquitoes, between the eleventh and the twelfth days. These intervals are used as the basis for separating the tabulated material into three categories: i.e., 1–2 days (filariform), 3–11 days (sausage-shaped), and 12–16 days (infective-stage). In only one mosquito, *C. sitiens*, were a few infective-stage larvae seen out of the Malpighian tubules by the eleventh day. One *A. pandani* contained some live but degenerating, sausage-shaped larvae on the twelfth day.

DISCUSSION

The data presented here show the relative efficiency of six species of mosquitoes in developing larvae of *Dirofilaria immitis*. It has not been possible to gather information suitable for evaluating accurately the mosquitoes as transmitting hosts. This would involve a study of the habits and the rates of infection of mosquito hosts in the "wild." The study of natural infections might be misleading due to the probable presence of other species of filaria in either wild or domestic animals.

SUMMARY

Six species of mosquitoes were studied on Guam, M. I., to determine their relative efficiency as developmental hosts for *Dirofilaria immitis* (Leidy). They were as follows: *Aedes guamensis* Farner and Bohart, *A. pandani* Stone, *Culex annulirostris* Skuse, *C. quinquefasciatus* Say, *A. aegypti* (Linn.), and *C. sitiens* Wied. The number of infective-stage larvae developing in from 25 to 120 specimens of these species of mosquitoes averaged 5.5, 2.3, 0.6, 0.4, 0.2, and 0.2, respectively. The respective percentages of the mosquitoes infected with infective-stage larvae were: 68, 57, 27, 23, 2, and 15.

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THE NEMERTEAN *CARCINONEMERTES* AS AN INDICATOR OF THE SPAWNING HISTORY OF THE HOST, *CALLINECTES SAPIDUS*[1]

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In the course of biological studies of *Callinectes sapidus* Rathbun, the blue crab or common commercial crab of Chesapeake Bay, notes were made on the parasitic nemertean *Carcinonemertes carcinophila* (Kölliker, 1845). These data have revealed some facts which contribute to an understanding of the biology of both the host and the parasite.

Humes (1942) described the morphology and the life cycle of *Carcinonemertes carcinophila*. This nemertean lives on the gills of female crabs, but becomes sexually mature only on the sponge or egg mass carried by the crab during the spawning period. During the period that the worms are on the sponge they feed on the eggs of the host. Humes stated that the worms probably return to the gills after all of the eggs of the host have hatched, basing this belief on his finding, in Louisiana, that 35% of the non-ovigerous females have worms on their gills while only 15% of the ovigerous crabs have infested gills. However, Humes was unable to distinguish non-ovigerous crabs which had already spawned from mature females which had not yet laid their eggs.

Humes examined 529 mature female crabs (*Callinectes sapidus*) during his study of *Carcinonemertes* at Grand Isle, Louisiana. I have data on 1011 mature female crabs in Virginia waters. As these crabs were examined primarily to determine the condition of their reproductive organs and their spawning history, these data are presented in such a way as to show the correlation between the reproductive cycles of the host and the parasite.

Hard (1942) described five stages in the reproductive cycle of the mature female crab. Newly inseminated crabs, whose seminal receptacles are swollen full of the semen deposited by the male and whose ovaries have not yet enlarged, are designated Stage I. In Stage II, the seminal receptacles are no longer swollen and the ovaries are larger. In Stage III, the ovary has reached full size but the crab has not yet laid any eggs. After spawning, empty egg cases or egg stalks can be found on the swimmeret hairs, even several months after the spawning season; crabs with eggs or remnants of eggs on the swimmerets are designated Stage IV if the ovary is still large and full of eggs, and Stage V if the ovary is exhausted. In the following table, Stages II and III are combined, as are Stages IV and V, because there was no difference in their nemertean infestations.

In the Virginia waters of Chesapeake Bay crabs begin to spawn or produce the sponge about the first of May, and sponge crabs are abundant in the lower Bay until some time in September. Each sponge or egg mass lasts about two weeks (the average time required from time of egg-laying to hatching) but it is probable that individual crabs spawn two or more times during the summer. During the fall the old spawned females are replaced by a new generation of females which have become

Received for publication, December 30, 1946.

[1] Contribution from the Virginia Fisheries Laboratory of the College of William and Mary and Commission of Fisheries of Virginia, Number 26.

TABLE 1.—*The percentages of mature female crabs* (Callinectes sapidus) *in various stages of the spawning cycle, which have nemerteans* (Carcinonemertes carcinophila) *on the gills. All data are for Virginia waters of Chesapeake Bay, from Mobjack Bay to Cape Henry, and York and James Rivers*

	Stage I buckram			Stage I hard			All Stage I	Stage II–III			Sponge			Stage IV–V		
	No. exam.	No. infes.	% infes.	No. exam.	No. infes.	% infes.	% all Stage I infes.	No. exam.	No. infes.	% infes.	No. exam.	No. infes.	% infes.	No. exam.	No. infes.	% infes.
July 1944	0	0	0	28	4	14.6	14.6	74	11	14.9	110	56	19.7	67	67	100.0
Aug. 1944	161	36	22.4	101	30	29.7	25.2	62	35	56.4	146	126	86.3	58	56	96.6
Sept. 1944	27	11	40.7	47	46	97.9	77.0	16	14	87.5	0	0	..	7	7	100.0
Total, Summer '44 ...	188	47	25.0	176	80	45.4	34.9	152	60	39.5	256	182	71.1	132	130	98.5
Winter '45–'46	..	..	..	..	..	..	..	99	96	97.0	..	..	..	8	6	75.0
Grand total	(All Stage I : 364 exam., 127 infest.)						34.9	251	156	62.2	256	182	71.1	140	136	97.2

mature during late summer and fall. In winter, only about five per cent of the mature females in commercial catches have the aged appearance and the empty egg-cases on the swimmerets which indicate that they have spawned during the previous summer; these are the Stage IV crabs of Hard; the predominant winter type is Stage III.

As shown by this table, Stage I has the lowest percentage of infestation by nemerteans. This is to be expected since these crabs had shed only a short time before, and Humes found that all nemerteans on the gills are left in the shed (cast exoskeleton) after the shedding process.

Stages II and III have only slightly higher percentages of infestation than Stage I during the summer, since many of the crabs in this stage are not much older than some of the Stage I crabs. By winter, however, nearly all of the Stage III crabs are infested.

An important point not shown by the table is the fact that all of the worms on the gills of Stage I, II, and III crabs are small, sexually immature, and either white or slightly pinkish in color, while most of the worms on crabs which have spawned are larger and bright red in color.

Stage IV and V crabs, which have already spawned at least once, nearly all have red worms on their gills regardless of the time of year, but the worms decrease in number by late winter.

The percentage of infestation of unspawned females and sponge crabs tended to increase during the spawning season, as would be expected from the fact that nemerteans were reproducing throughout the crab spawning season and thus producing an increasing number of infective larvae. Even Stage I crabs showed an increased infestation, in spite of the fact that during August and September the majority of these had shed so recently that their shells were not yet completely hard—that is, they were still in the so-called "buckram" condition.

These data, together with observations on the reproductive stages of the nemerteans on the crab sponges, tend to substantiate Humes' theory that *Carcinonemertes* migrates from the gills to the crab's egg mass to become sexually mature and lay its own eggs, then moves back to the gills. Furthermore, they seem to indicate that the infestation of new hosts occurs not only during the crab spawning season but during the fall, and that both mature and immature worms live over winter on the gills of dormant crabs in lower Chesapeake Bay.

Through the courtesy of Miss Rosalie Rogers of the Virginia Fisheries Laboratory, data on the percentage of *Carcinonemertes* infestation in the egg mass of sponge crabs collected each week during the spawning season of 1944 are shown in Fig. 1. The percentages of crabs in various stages of the reproductive cycle which had *Carcinonemertes* on the gills are shown for each week throughout the latter half of the spawning season, July 9 through the week of September 3, 1944. Unfortunately the gills were not examined for nemerteans during the first half of the season. It may be noted that nemertean infestations both in the sponge and in the gills were greatest during August and September, and that they reached the highest peak near the end of the spawning season in all types of mature female crabs.

In York River and adjoining waters the percentage of nemertean infestation on crabs which had not spawned was lower in July and August (14 and 28 per cent) than it was in the region from Hampton Roads to Cape Henry (50 per cent in July,

85 in August). In September, however, over 90 per cent of the unspawned crabs were infested in all waters, even those where sponge crabs are very rarely seen (upper York River and James River). From 94 to 100 per cent of the crabs which had spawned were found infested in all waters throughout the period July to September.

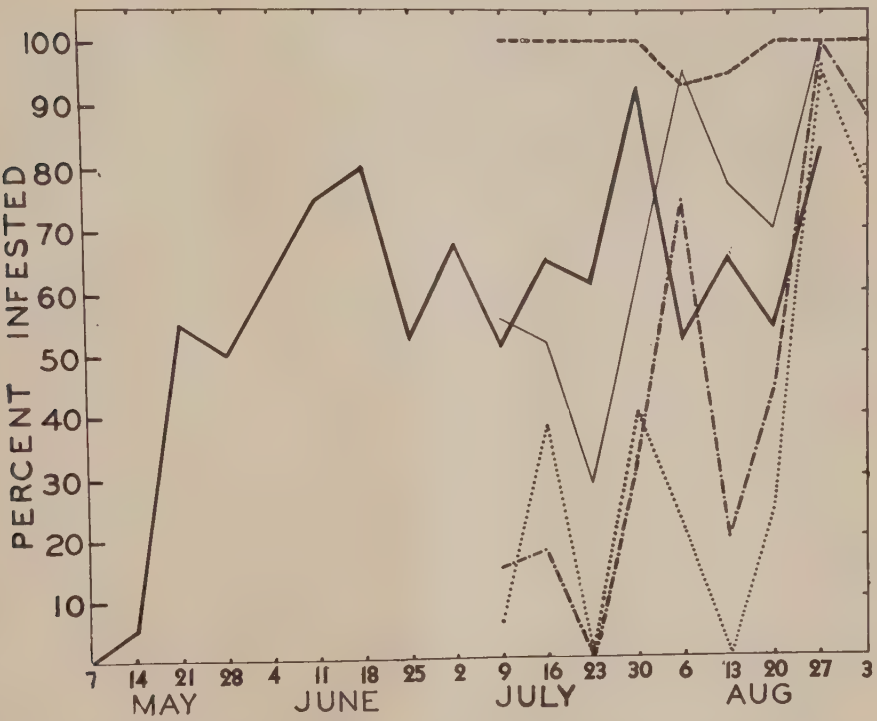

FIG. 1. Percentage of mature female crabs in Virginia waters of Chesapeake Bay infested by *Carcinonemertes carcinophila* each week of 1944 spawning season. Dates are first days of weeks during which samples were taken. Heavy, solid line represents percentage of sponge crabs which had nemerteans on the egg-mass. Heavy broken line is percentage of non-ovigerous crabs, already spawned, whose gills contained large red nemerteans. Light solid line is percentage of sponge crabs which had either light-colored or red nemerteans in gills (all but 4 of 182 infested crabs had red worms). Dot and dash line represents percentage of Stage II and Stage III females (with eggs developing in ovary but not yet spawning) whose gills contained small light-colored nemerteans. Dotted line represents Stage I crabs (recently inseminated, ovary still thread-like) with small white nemerteans on gills. Gills not examined for nemerteans prior to July 9.

Of greatest practical interest is the fact that non-ovigerous female crabs which have already spawned once can be distinguished from non-spawning females by examination of the gills with the unaided eye. If large red worms are seen on the gills, the crab has spawned some time in the past. If no worms, or only small white worms are seen, the crab has probably never spawned. The data so far indicate this method to be about 97 per cent accurate.

SUMMARY

1. Only immature, light-colored *Carcinonemertes* are found in the gills of mature female crabs (*Callinectes sapidus*) which have never spawned.

2. Large, red *Carcinonemertes* are found only in the gills of mature female crabs which have spawned at least once, or in the gills and egg-mass (sponge) of spawning females.

3. The finding of large red nemerteans in the gills, by inspection with the naked eye, is a sure sign that the crab has spawned some time in the past. About 97 per cent of the crabs which have spawned, in lower Chesapeake Bay, may be detected by this easy method.

4. The percentage of mature female crabs infested by *Carcinonemertes* tends to increase throughout the summer. Over 95 per cent of all mature female crabs retain nemerteans in their gills throughout the winter, in lower Chesapeake Bay.

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SARCOCYSTIS IN *MACACA MULATTA**

I. N. Dubin and Aimee Wilcox

Since Miescher's discovery of Sarcosporidia in mice in 1843, this type of parasite has been found in many other animals. In the great majority of cases, infection with *Sarcocystis* produces no ill effects, but there is evidence to indicate that occasionally the parasite may cause serious damage (Wenyon, 1926; Scott, 1930). Its presence in *Macaca mulatta* was first noted by De Korté (1905). Since that date we have been able to find only one other report of *Sarcocystis* in the monkey, namely, an abstract by Offutt and Telford (1945). Offutt and Telford noted that their animals suffered from "cage paralysis" and while they did not ascribe these symptoms to the presence of the parasite they raised the question whether or not there was any relationship between the paralysis and the sarcocysts.

In view of the rarity of the reported cases of *Sarcocystis* in the monkey and the problem of pathogenicity of the parasite in this animal, we are reporting our observations in three rhesus monkeys in which this parasite was found.

OBSERVATIONS

The parasites were discovered incidentally in 3 of 4 monkeys which were examined histologically in the course of malarial studies. Prior to inoculation with *Plasmodium cynomolgi* the monkeys appeared healthy, and it seems that the presence of *Sarcocystis* in these animals had produced no ill effects.

The animals were killed with sodium pentobarbital and the tissues immediately fixed in formalin-Zenker fluid. No abnormalities were noted on gross examination of the tissues at autopsy. Paraffin sections were stained with Maximow's method (Cowdry, 1943).

Although complete autopsy studies were made on these animals, the parasites were seen only in the histological preparations of the myocardium and skeletal muscles.

Parasites in different stages of development were seen. Each cyst was surrounded by a thin capsule. No septa, such as have been reported by many observers, were seen within the cysts. The morphology of the younger forms is quite distinct from that of the older ones (Figs. 1, 4, 5, 6). The smaller cysts were spherical, while the larger ones became more spindle-shaped as they replaced more and more of the original mass of the muscle fiber.

The smallest cyst found measured 16.7 microns in diameter and contained numerous sporozoites. The sporozoites in the smaller (and presumably the younger) cysts were more loosely spaced than in the larger ones and took the stain less intensely. The individual sporozoites in the smaller cysts measured about 3 microns, the nucleus measuring about 1.5 microns. The cells were roughly circular; the nuclei were round or oval and were eccentrically placed; the cytoplasm was pale-staining and the outlines often indistinct; frequently a vacuole was seen immediately adjacent to the nucleus. The cells at the periphery of the cyst were more tightly packed than those in the center.

Received for publication, December 13, 1946.

* From the Division of Pathology and Bacteriology, University of Tennessee, and the Office of Malaria Investigations, U. S. Public Health Service, Memphis, Tennessee. Supported in part by a grant-in-aid from the Tennessee Valley Authority.

In cysts of intermediate size (about 30 microns in diameter) the sporozoites were crescent-shaped and measured about 4.5 to 5 microns in length.

In the largest cysts the sporozoites were more elongated, although still crescentic, and measured about 6 to 7.5 microns in length and 1.5 microns in transverse diameter.

The longest cyst measured 360 microns in length. The widest cyst measured 84 microns in transverse diameter and lay in a muscle fiber which had a diameter of 102 microns. The variation in the size of the cysts is seen in Fig. 2.

Each cyst was restricted to the boundaries of a muscle fiber. We did not see any cysts which had grown beyond the limits of the muscle fiber, such as has been reported by others.

The only lesions noted were replacement of the muscle fibers by the parasite and a rare focus of infiltration by inflammatory cells (Fig. 3). The parasites were seen in the myocardium, the diaphragm and the psoas muscles; the latter two were the only skeletal muscles examined.

DISCUSSION

The source of infection in these animals is unknown to us. Nor do our observations throw any light on the life cycle of the parasite, except for the differences in morphology between the younger and older forms.

It seems quite clear that the pathogenicity of the parasite for the monkeys was negligible in spite of a marked degree of infection in one animal. The animals seemed healthy; furthermore, there was practically no inflammation in response to the presence of the cysts. Rarely, a muscle fiber containing a cyst was surrounded by a small collection of cells, chiefly mononuclear, although a few eosinophils were also present. The inflammatory cells were always at the periphery of the parasitized muscle fiber and were never seen immediately adjacent to the cyst itself. It appears that this inflammation was in response to the damaged muscle tissue rather than to the parasite; the irritating effect of damaged muscle is well known.

SUMMARY

Sarcocystis infestation of monkeys discovered incidentally at autopsy is described. The cysts were found only in the myocardium and the skeletal muscles. The differences in the morphology of the younger and older forms of the parasite are noted. The pathogenicity of the parasites for the monkeys was negligible; inflammation was rare and when it occurred was in response to the damaged muscle tissue rather than to the parasite itself.

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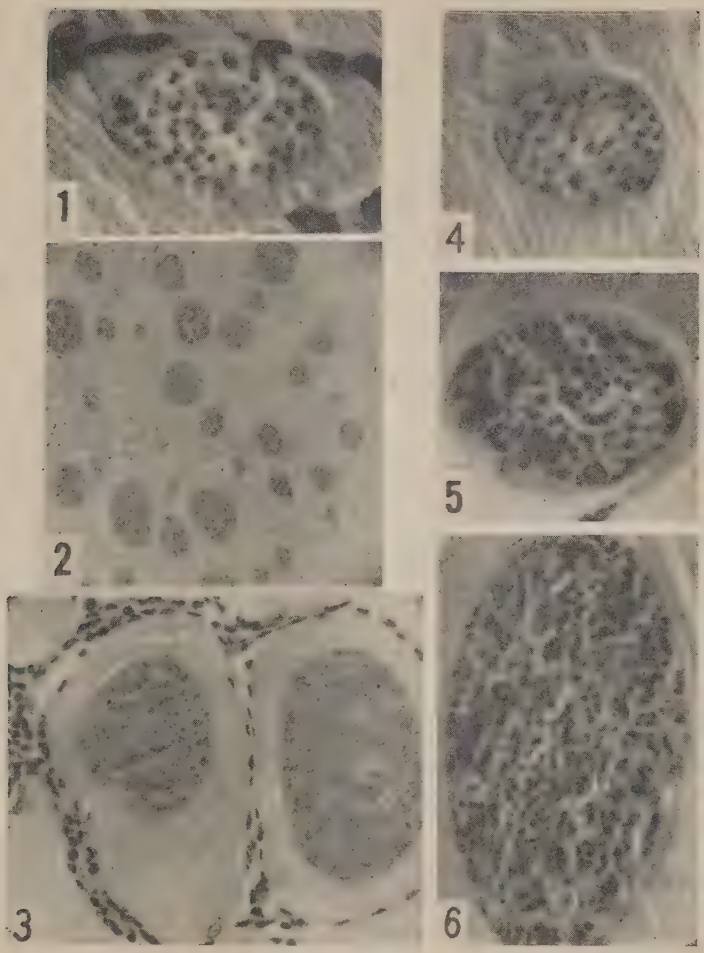

EXPLANATION OF PLATE

(Photomicrographs were taken by Clinton S. Smith, U. S. Public Health Service, Memphis, Tennessee)

Tissues were fixed in formalin-Zenker fluid; sections were stained with Maximow's stain.

FIGS. 1, 4 and 5. Young sarcocysts. The sporozoites in Figs. 1 and 4 are roughly circular while those in Fig. 5 are more crescentic. (970×.)

FIG. 2. A low power view of psoas muscle to show the variation in size of the cysts, as well as the marked degree of infestation. (100×.)

FIG. 3. Collection of inflammatory cells at periphery of two adjacent parasitized muscle fibers. (430×.)

FIG. 6. Older sarcocyst. On comparison with Figs. 1, 4 and 5 note that the sporozoites are now larger, more elongated and take the dyes more intensely. (970×.)

THE RATE OF MULTIPLICATION OF *ENDAMOEBA HISTOLYTICA* AND ITS RELATION TO *IN VITRO* DRUG TESTING AND POSSIBLY TO NUTRITIONAL STUDIES*

STERLING BRACKETT AND ALEXANDER BLIZNICK

The high degree of activity of metachloridine (2-metanilamido-5-chloropyrimidine) in gallinaceum malaria (*Plasmodium gallinaceum*) in chickens (Brackett and Waletzky, 1946) led to its trial against *Endamoeba histolytica in vitro*. Other compounds showing antimalarial activity were likewise tested against *E. histolytica* and this paper records the results.

Metachloridine, as shown by earlier work (Brackett and Waletzky, 1946), does not bring about the complete inhibition of development and multiplication of *P. gallinaceum* before three or four nuclear divisions have taken place. This same relatively slow rate of action had been demonstrated with the action of sulfadiazine (Brackett, Waletzky and Baker, 1945) on *P. gallinaceum* and with various sulfanilamide derivatives on bacteria (Henry, 1943; Rose and Fox, 1942; Libby, 1940). The initial action of such compounds is unquestionably inhibitory rather than lethal. Although the commonly used procedures for cultivating *E. histolytica* (Rees and Reardon, 1945) and for drug testing with this organism (Anderson and Chuan, 1944) are adequate for demonstrating lethal action they were examined critically since it was felt that unless they permitted the demonstration of inhibitory drug action they would not be suitable for testing metachloridine and similarly acting compounds. The practical value of an amebastatic drug, of course, is dependent on its being able to give complete cures either with the aid of the host's normal defense mechanisms or by prolonged treatment—possibilities which are discussed in greater detail later in the paper.

It is commonly stated that large inocula are necessary to insure successful cultures of *E. histolytica*. In fact the usual practice is to subculture half the sediment of a tube. Since most of the amebae are concentrated in the sediment, the inoculum must contain not much less than half of the total population of a culture tube. Under such conditions there could be only a two- or three-fold increase in ameba population or one or two divisions of the amebae in the daughter tube. By reducing the number of amebae in the inoculum, sometimes very markedly, as much as a 35,000-fold increase in amebae has been observed in a single tube. This increase could only be accounted for by not less than 15 multiplications. The average fold increase in the untreated controls in many drug testing experiments indicated that about six divisions took place under the conditions used.

GENERAL METHODS

The strain of *Endamoeba histolytica* used was the NIH 103 strain obtained from Dr. C. W. Rees of the National Institute of Health, Bethesda, Maryland. This strain was in association with a single species of bacterium referred to by Dr. Rees and his co-workers as the "t" organism. This combination of ameba and associate

Received for publication, January 10, 1947.

* From the Chemotherapy Division of the Research Laboratories of the American Cyanamid Company, Stamford, Connecticut.

has been maintained by use of sterile technique in transferring the cultures at alternating three- and four-day intervals using about half of the sediment of a doner tube to inoculate a new tube. The "t" organism is a microaerophilic, spore-forming, pleomorphic, gram-negative rod which produces large quantities of hydrogen from carbohydrates (Rees et al, 1942). Using this combination of *E. histolytica* with a single species of bacterium eliminates at least one important variable which is encountered when a mixed bacterial flora of unknown and fluctuating composition is used.

The medium used was a whole egg slant overlain with Locke's solution to which a small quantity of sterile rice flour was added at the time of inoculation (Reardon and Rees, 1939). Test tube cultures were used throughout with about 5 to 7 cc of egg-Locke's mixture coagulated into a slant without a butt, and exactly 10 cc of Locke's solution used as the overlay. The Locke's solution was modified by a reduction in the amount of $CaCl_2$ to 0.01 g per liter of solution. This gives a precipitate-free solution without the necessity of boiling and filtration. The tubes were autoclaved at about 9 pounds of steam pressure for thirty minutes and checked for sterility by overnight incubation at 37° C.

Amebae were counted by methods proposed by Paulson (1932) and Craig (1939) slightly modified. In this procedure the cotton plug of a tube is replaced by a sterile rubber stopper and the sediment thoroughly suspended in the fluid overlay by shaking. A sample of this suspension is taken and put into a Spencer bright-line improved Neubauer haemocytometer and the number of amebae in all 9 large squares counted. Multiplication of this figure by 10/9 gives the number of amebae per mm^3.

MULTIPLICATION RATE OF *Endamoeba histolytica*

Known numbers of amebae to be used for inoculations of graded sizes were obtained by using measured amounts of the undiluted suspension whose amebae count had been determined, or by using measured amounts of a diluted suspension, or if very small numbers were desired, a standard 3-mm bacteriological loop was used to pick up small quantities of a homogeneous suspension. In general, 0.1 cc of suspension was used. No total counts of the amebae in such samples were ever made so the extent of variation in the number of amebae from one 0.1 cc sample to another is not known. Before using a loopful of suspension as an inoculum, counts were made on the number of amebae per loop. Variation from loop to loop is illustrated by the following series of counts: Tube A, 47, 50, 55, 45, and 60 amebae per loop successively; tube B, 15, 16, 31, 71, 35, 20, 23, 15, 13, 15, 20, and 18; Tube C, 65, 52, 48, 62, and 65; tube D, 17, 16, 13, 15, and 19 amebae per loop.

When less than several hundred amebae were used to inoculate each tube, not all tubes became infected (Table 1). With somewhat larger inocula, but inocula still much smaller than generally considered necessary, the tubes invariably developed

TABLE 1.—*Relationship between the number of amebae* (E. histolytica) *used as an inoculum and the proportion of culture tubes becoming positive*

No. amebae in inoculum	No. of trials	Tubes positive / Tubes inoculated
15–20	6	63/93
50–60	2	26/42
125	1	4/4
400	2	34/35
500	2	10/10
1,000–5,000	17	118/118

good populations of amebae. Reasons for the failure of amebae to grow out in all of the tubes inoculated with smaller numbers are not known. The results were somewhat variable in different tests. In three of the six tests inoculated with 15 to 20 amebae, all of the 22 tubes became positive. In the poorest test only 4 out of 11 tubes became positive.

If the amebae grew at all the total numbers in each tube were on the average about the same regardless of the size of the inoculum (Table 2). In fact as many or more amebae were harvested in tubes inoculated with 15 or 20 amebae as were harvested on the average from passage tubes inoculated with half the sediment of another passage tube or anywhere from 100,000 to 250,000 amebae although the latter counts may not have been made at the peak of the growth curve. As would be expected, the time required to reach these maximum populations is dependent on the number of amebae used to start a culture. The data on fold increase in amebae populations

TABLE 2.—*Increase in populations of* E. histolytica in vitro *when different numbers of amebae are used in the inoculum*

No. of tubes	Amebae per tube (in thousands)			Fold increase	Calculated number of divisions	Duration of trial in days	Mean no. divisions per day
	At outset	At end					
		Range	Mean				
4	0.015	500– 600	550	36,000	15	6	2.5
14	0.020	45–1,300	550	27,000	14.5	6	2.5
5	0.050	50– 350	200	4,000	12	6	2
5	0.050	10– 200	45	900	10	6	1.75
8	0.060	200– 500	400	7,000	13	6	2
4	0.125	50– 300	120	1,000	10	4	2.5
8	1.5	10– 500	140	95	6.5	3	2
4	2.0	400– 500	450	225	8	4	2
8	2.0	70– 880	350	175	7+	3	2
7	2.0	40– 210	107	50	5+	3	2
8	2.2	50– 410	270	125	7	3	2
6	2.3	60– 230	160	70	6+	3	2
8	2.5	45– 80	60	25	4.5	3	1.5
8	3.0	55– 650	260	85	6+	3	2
8	3.5	45– 280	135	40	5	3	2
10	3.8	33– 680	260	70	6+	3	2
11	4.0	20– 180	100	25	4.5	3	1.5
8	5.0	80– 510	260	50	5+	3	2
8	5.2	120– 970	430	80	6+	3	2

given in Table 2 can be used to calculate the approximate multiplication rate by assuming binary fission without mortality. On this basis the approximate number of divisions taking place in each set of experimental tubes is indicated (Table 2) and this varies from 15 to 4.5 as the number of amebae in the inoculum increases from 15 or 20 to 4,000 to 5,000. Dividing the average number of cell divisions by the time required for this multiplication, it is evident that under the particular conditions of these experiments the amebae multiply at the rate of about 2 to 2.5 divisions a day. It may seem to be somewhat less than this in tubes inoculated with 2,000 to 5,000 amebae but this may simply be due to the fact that the maximum populations were reached somewhat before the seventy-second hour, the time arbitrarily accepted for the examination of these cultures. If the mortality rate were known and taken into account and if the calculations were made from data taken only during the logarithmic phase of the curve the rate of multiplication would undoubtedly be higher than the minimum figure given here. So far as we can determine, this is the first report which specifically expresses the growth rate of cultures of *E. histolytica* in terms of numbers of divisions per day. Other such reports would be valuable for comparative purposes. Calculations made from the data given in the recent paper

of Balamuth and Howard (1946) indicate the multiplication rate under their conditions to be about 2.5 divisions a day which is in line with our figures.

The rather extensive range in amebae counts at the end of cultivation (Table 2) cannot be accounted for by any of our data or observations and needs further investigation. Similar variation has also been noted by others (Rees et al, 1944) who have cultured *E. histolytica* with the single species of bacterium designated as the "t" organism.

DRUG ACTION

a. *Inhibitory or Amebastatic Action*

Since studies of drug action were, for the sake of expedition, started before the studies of the effect of inoculation size were completed, it was necessary to choose an arbitrary number of amebae to use for starting cultures. This was 0.1 cc of a homogeneous suspension of amebae from a seventy-two hour stock culture tube or approximately one one-hundreth of the total number of amebae in a stock culture tube. In terms of actual numbers, the inocula varied from 1,500 to 5,000 amebae. Much of the data in Table 2 was taken from the controls of these drug trials.

Compounds to be tested were dissolved, if soluble, in the fluid overlay of the culture tubes before sterilization by autoclaving. If there was any reason to suspect that the compound would be altered by heating, the drug solution was sterilized by filtration and added aseptically to sterile egg slants. Insoluble compounds were made up as suspensions and distributed into tubes as such. Of course the material settled on standing to give higher concentrations on the surface of the egg slant. These compounds are presumably ingested by the amebae. In each case the compounds were tested at a minimum of four concentrations usually starting with 100 mg/100 cc and dropping by two-fold dilutions. Results were read by microscopic examination of the sediment after seventy-two hours of cultivation. A tube was recorded as negative if no amebae were seen in ten fields (10 × oculars; 43 × objective) and positive if one or more were seen. The set of dilution tubes for each compound was controlled by an untreated tube and the amebae population in this tube was counted at the end of the trial. If amebae multiplied well in the control tube but not in the treated tubes, further dilutions of the compound were made and retrials run until the minimum effective (or inhibitory) concentration was determined. Our results with two standard compounds, namely, emetine and vioform are given in Table 3. These two compounds were used in every trial of new compounds and serve

TABLE 3.—*Titration of amebastatic activity of emetine and vioform**

Compound	Conc. mg/100 cc	Amebae growth in separate tests†							
		2	4	5	6	7	8	9	10
Emetine	2	−	−	−	−	−			−
	1	−	−	−	−	−	−	−	−
	0.5	+	−	+	−	−	+	−	+
	0.25	+	−	+	+	+	+	+	+
	0.125						+	+	
Vioform	2	−	−	−	−	−			−
	1	−	−	−	−	−	−	−	−
	0.5	+	−	−	−	−	+	−	+
	0.25	+	−	−	−	−	+	−	+
	0.125						+	+	

* Inoculum 1,500–5,000 amebae. Seventy-two-hour endpoint.
† In all cases good growth was obtained in untreated tubes.
+ = amebae readily found in sediment.
− = no amebae in 10 high power fields.

TABLE 4.—*Compounds tested for* in vitro *amebastatic activity* (Endamoeba histolytica)

Number	Name	Reference or Source	Amebastatic endpoint mg/100 ml
I	p-Aminobenzoic acid	Eastman Kodak Company	½
II	3,5-Dibromo-2-aminobenzoic acid	13	2
III	Sodium 3,5-diiodobenzoate	14	4
IV	2-n-Heptyl-4,6-diamino-s-triazine	American Cyanamid Company Stamford Laboratories	4
V	2-n-Heptyl-4-amino-6-hydroxy-s-triazine	"	4
VI	2,4-Diamino-6-octyl-s-triazine	"	4
VII	2,4-Diamino-6-n-nonyl-s-triazine	"	$< 12 > 4$
VIII	2-p-Nitrophenyl-4,6-diamino-s-triazine	"	sat (< 8)
IX	2-β-n-Butylaminoethyl-4,6-diamino-s-triazine	"	16
X	2-β-Cyanoethyl-4,6-diamino-s-triazine	"	16
XI	2-n-Octyl-4-amino-6-hydroxy-s-triazine	"	> 8
XII	2-(1-Bromo-2-methylpropyl)-4,6-diamino-s-triazine	"	> 16
XIII	2-(3-Heptyl)-4,6-diamino-s-triazine	"	> 16
XIV	2-Methyl-4,6-diamino-s-triazine	"	> 16
XV	2-β-Oximinocarbamylethyl-4,6-diamino-s-triazine	"	> 16
XVI	2-β-Carbamylethyl-4,6-diamino-s-triazine	"	> 16
XVII	Sodium β-(2,4-diamino-6-s-triazinyl) propionate	"	> 16
XVIII	2-(2-Acetylaminoethyl)-4,6-diamino-s-triazine	"	> 16
XIX	2,4-Diamino-6-trichloromethyl-s-triazine	"	> 16
XX	2-n-Amyl-4,6-diamino-s-triazine	"	100
XXI	2-(N-acetyl-β-n-butylamino-ethyl)-4,6-diamino-s-triazine	"	> 100
XXII	2-n-Hexyl-4,6-diamino-s-triazine	"	> 100
XXIII	2-Methyl-4-amino-5-bromo-methylpyrimidine hydrochloride	Eastman Kodak Company	1
XXIV	7-Methylguanine	8	12
XXV	5-Amino-7-hydroxy-1-v-triazolo (d) pyrimidine	11	25
XXVI	2-Methyl-4-hydroxy-5-ethoxy-methylpyrimidine	2	100
XXVII	2-Methyl-4-chloro-5-ethoxy-methylpyrimidine	2	100
XXVIII	2-Sulfanilamido-5-iodopyrimidine	12	1
XXIX	2-Sulfanilamido-4-amino-5-bromopyrimidine	6	1
XXX	N^1-(3,5-Dibromophenyl)-sulfanilamide	9	½
XXXI	2-Sulfanilamido-5-chloropyrimidine	6	1
XXXII	Sulfadiazine	10	1
XXXIII	Mapharsen (oxophenarsine hydrochloride)	Parke, Davis and Company	⅛
XXXIV	p-Arsenosophenoxyacetamide	4	12
XXXV	Dimethylammonium dimethyldithiocarbamate	American Cyanamid Company Stamford Laboratories	25
XXXVI	Cupric dimethyldithiocarbamate	"	5 mg/100 cc suspension
XXXVII	2-Metanilamido-5-chloro-pyrimidine	7	100
XXXVIII	2-(6-Methylmetanilamido)-5-chloropyrimidine	7	100
XXXIX	2-Metanilamido-5-iodopyrimidine	7	> 100
XL	2-Benzenesulfonamido-5-chloropyrimidine	7	100
XLI	Acetate salt of 1-p-chlorophenyl-5-isopropyl biguanide	3	100
XLII	d(+)Pantoyltauryl-3,5-dibromoaniline	15	100
XLIII	d(+)Pantoyltauranilide	15	200
XLIV	Quinacrine (Atabrine HCl)	Winthrop Chemical Company Inc.	2
XLV	Quinine HCl	Merck & Co., Inc.	50
XLVI	Pamaquin (Plasmochin naphthoate)	Winthrop Chemical Company Inc.	100
XLVII	Chiniofon	Winthrop Chemical Company Inc.	100
XLVIII	Diodoquin	G. D. Searle & Company	100 mg/100 cc suspension
XLIX	2-Thiouracil	1	100
L	α-Bromopropionic acid	Eastman Kodak Company	> 100
LI	γ-(3,4-Ureylenecyclohexyl) butyric acid	5	> 100

as a basis for comparison of the relative activity of these compounds. The minimum effective concentration (i.e., the smallest amount of drug that suppressed amebae growth for three days) of emetine was from 0.25 to 1.0 mg per 100 cc in different tests, while vioform may possibly be a little more active. For purposes of comparison the M.E.C. of these two compounds in this particular testing procedure is considered to be about 0.5 to 1.0 mg per 100 cc.

Table 4 gives the results of the trials of other compounds in this amebastatic test. The compounds have been roughly grouped by chemical structure in this table and the groups placed in the general order of their activity and interest. The activity is expressed in terms of the minimal inhibitory concentration with no attempt to express the activity as a ratio of that of the standards, since these trials do not necessarily indicate whether the compound has a direct action on the amebae or an indirect action through on inhibitory effect on the "t" organism associate. The more active compounds in this test warrant further special investigations to determine whether the action is direct or indirect.

b. *Direct vs. Indirect Drug Action*

Attempts were made to devise a simple procedure that would show whether a compound is inhibitory to the "t" organism without the necessity of running separate bacteriostatic tests. Although the "t" organism normally produces large amounts of gas, detectable with gas tubes, this was not consistent enough to serve as a criterion of good bacterial growth. However, it was found that visual turbidity could be used as a rough indication of the extent of growth even though the growth of the "t" organism is less luxuriant than that of many other species of bacteria. This is illustrated by an experiment in which visual turbidity was compared with bacterial counts made by the dilution count procedure. The "t" organism was introduced into tubes of regular medium for *E. histolytica* containing in most cases several times the amebastatic concentrations of a number of the most active compounds from Table 4. After cultivation for sixteen hours the tubes were checked for visual turbidity and, in addition, 1.0 cc of the fluid from a tube of each compound was inoculated into 9.0 cc of Brewer's thiogycallate medium. Serial dilution in ten-fold steps was made in Brewer's medium in order to determine the bacterial concentration in the original drug treated tube. The results are given in Table 5. The results indicate that populations of the "t" organism in this medium after 16

TABLE 5.—*Inhibitory effect of various compounds on the "t" organism in Brewer's thiogycallate medium*

Compound	Conc. mg/100 cc	Turbidity at 16 hours	No. organisms* per cc at 16 hours
Untreated controls		+,+,+	10^7
2-n-Heptyl-4,6-diamino-s-triazine	4	+,+	10^7
2-Methyl-4-amino-5-bromomethyl-pyrimidine hydrochloride	4	+,+	10^8
p-Aminobenzoic acid	4	+,+	10^7
2-Ethyl-4,6-diamino-s-triazine	8	+,+	10^8
Sulfadiazine	4	0,0	10^4
Emetine	2	+,+	10^7
Vioform	2	0,0	10^6
Chiniofon	25	0,±	10^7

* Based on a dilution count procedure using ten-fold steps in diluting.
+ = definite turbidity.
± = questionable turbidity.
0 = no visible turbidity.

hours of cultivation are about 10 to 100 million organisms per cc. No further increases in turbidity with further cultivation suggest that these are the maximum populations to be expected. Since it is common experience that populations of somewhat less than 10 million organisms per cc may not be visible there is only limited possibilities for grading the growth of the "t" organism by this means. It is evident that growth to the point of visual turbidity is sufficient to permit amebae growth, other conditions permitting. The absence of visual turbidity may or may not indicate insufficient bacterial growth to permit amebae growth and the point can only be determined by making a count of the bacteria by methods such as the serial dilution procedure. The inhibition of amebae multiplication in the sulfadiazine-treated tube indicates that 10,000 bacteria per cc may be insufficient for amebae growth although the drug may be acting directly on both the amebae and the "t" organism.

Compounds inhibitory to the "t" organism may or may not be inhibitory to the amebae but unless the minimum effective concentration for the "t" organism is higher than for the amebae, other procedures are necessary to prove the direct action of the drug on the amebae. One possibility would be to culture the amebae with other species of associated bacteria. Inhibition of amebic growth without accompanying inhibition of bacterial growth would be conclusive evidence of a direct action of the drug on the amebae.

Sulfadiazine was not tested in this way because it was found that the "t" organism would eventually produce turbid cultures at drug concentrations at least four- to eight-fold higher than those inhibiting amebae growth in the seventy-two-hour test. In other words, the drug simply reduced the multiplication rate of the "t" organism but did not destroy it or completely inhibit its multiplication. When ameba cultures treated with these concentrations of sulfadiazine were held and examined at one hundred and twenty hours it was found that the cultures became turbid with "t" organism growth and many amebae could be found in the sediment. It seems probable that amebae introduced into culture media containing sulfadiazine do not begin to multiply extensively until the retarded population of "t" organisms finally reach certain concentrations. It is concluded that sulfadiazine and the other sulfanilamide derivatives tested act only indirectly against *E. histolytica in vitro*.

Amebae may grow out in a tube showing little or no turbidity since such a tube may actually have bacterial growth about equal to that of the untreated tubes as is illustrated by chiniofon at 25 mg/100 cc (Table 5) which is not inhibitory to amebae at 50 mg/100 cc (Table 4). It may be safely concluded then that all the compounds in Table 5, with the exception of sulfadiazine, act directly on the amebae since they were not significantly inhibitory to the "t" organism.

c. *Amebastatic vs. Amebacidal Action*

As already indicated, the commonly used drug testing procedures (Anderson and Chuan, 1944) determine only the amebacidal activity of compounds. The amebacidal concentration of vioform is reported as being 17 mg/100 cc (1 : 6,000) by Anderson and Chuan (1944). Emetine hydrochloride is reported by these same investigators to be amebacidal at 10 to 20 mg/100 cc (1 : 10,000 to 1 : 5,000) and by Dobell and Laidlaw (1926) to be 4 mg/100 cc (1 : 25,000). Anderson and Chuan also report the amebacidal concentration of mapharsen to be 3 to 5 mg/100 cc

(1:30,000 to 1:20,000). As is seen by reference to Tables 3 and 4 the effective amebastatic concentrations reported here for these three compounds are considerably lower. Although this was to be expected there was also a possibility that the strain used by us might be more or less sensitive to drug treatment in general than are the strains used by others. For sake of reference several amebacidal tests were conducted. Instead of introducing large numbers of amebae into solutions of the test compounds, we added the test compounds to tubes containing large numbers of amebae and then made examinations at intervals thereafter to determine the lethal action of the drugs. The results of these trials are given in Table 6 and are indicated by the widely used subjective procedure of multiple pluses. For key to these see

TABLE 6.—*Amebacidal action of several drugs on seventy-two-hour cultures of* E. histolytica

Compound	Conc. mg/100 cc	Amebae in sediment at time indicated after introduction of drug			Results of subinoculation*
		6 hours	24 hours	48 hours	
2-n-Heptyl-4,6-diamino-s-triazine	8	+++,+++	+++,+++	+++,++	
	25	+,+	+,0,0		+++,+++
2-Methyl-4-amino-5-bromomethyl-pyrimidine	8	+++,+++	+++,+++	+++,++	
	25	++,+	+,+,+		0,0
p-Aminobenzoic Acid	8	+++, +++	+++, +++	+++, +++	
	25	+++, +++	+++, +++		
Sulfadiazine	8	+++, +++	+++, +++	++, ++	
	25	+,+	++,+,++		
Emetine	8	+++, +++	++,++	+,+	
	20	+,+	+,+,+		0,0
Vioform	8	+++, +++	+++,+	0,0	
	25	++,++	+,+,+		0,0
Mapharsen	8	+++, +++	+++, +++	+,++	
	25	+,+	+,+,+		+++,+++

* Read at 96 and 120 hours after inoculation.
Key to symbols: +++ many amebae per high power field.
++ few amebae per high power field.
+ less than one amebae per field.
0 none in 20 to 30 fields.

table. Eight mg/100 cc of emetine and vioform caused the marked reduction of amebae in forty-eight hours but not twenty-four hours. This is about in line with the results of other investigators. Mapharsen may have been somewhat less effective in our hands than in the hands of Anderson and Chuan. It seems on the basis of these limited data that the ameba strain used by us is not strikingly different in drug sensitivity from those strains used by others.

Several other points of interest are brought out by Table 6. Even though mapharsen and 2-n-heptyl-4,6-diamino-s-triazine caused a marked reduction of amebae in twenty-four hours at concentrations of 25 mg/100 cc, there still remain some viable amebae as shown by subinoculation. On the other hand, even though some amebae could be found at twenty-four hours in 25 mg/100 cc solutions of 2-methyl-4-amino-5-bromomethylpyrimidine, vioform, and emetine, they were not viable in subinoculations.

DISCUSSION

There is no evidence available to indicate whether the drugs commonly used in clinical amebiasis are acting amebastatically or amebacidally. Since the compounds possess either type of action *in vitro,* depending on the concentrations, it is possible that they may act in either fashion in the host, again depending on the concentration of the drug at the site of action. Unfortunately, little or nothing is known concerning the distribution and concentrations of these drugs in different tissues or in the parasites.

The ability of an inhibitory drug to bring about complete sterilization of an infection would depend on whether the normal defense mechanisms of the host are able to destroy the inhibited parasites or whether these inhibited parasites would die if kept in a static condition for a sufficiently long period of time. The immunity in amebiasis is not clearly understood but the tendency for persons in an endemic area to be less susceptible to acute attacks suggests the development of some resistance, and there is no question that certain types of antibodies develop as is shown by the complement fixation test.

Assuming that an amebastatic drug might be of practical value then those compounds in Table 4 which are inhibitory at concentrations similar to the inhibitory concentrations of emetine and vioform are of considerable interest, since they may be as effective as these standard drugs but might be superior in respect to pharmacological and toxicological properties.

Probably the most interesting group is that which contains para-aminobenzoic acid (PABA). This compound (Table 4, I) is equal to emetine and vioform in amebastatic activity and its activity is almost surely directly on the amebae. Since the compound has already been used in man for other infections (Yeomans et al, 1944) trials in clinical amebiasis could be made without further investigations. If the drug showed activity in clinical amebiasis, related compounds should be investigated with the hope of finding a compound with better pharmacological properties. The activity of several related compounds (Table 4, II and III) indicates that the amebastatic activity is not restricted to PABA. The direct action of PABA on *E. histolytica* combined with the report of activity against *Pasturella tularensis* (Tamura, 1944) adds weight to the possibility that the compound is effective in typhus because of its direct action on the rickettsiae rather than because of an indirect action through the host.

None of the long series of triazines tested following the demonstration of activity in 2-n-heptyl-4,6-diamino-s-triazine (Table 4, IV) was more active than this compound and most of them were inactive. This particular triazine and also 2-methyl-4-amino-5-bromomethylpyrimidine (Table 4, XXIII) undoubtedly deserve a trial in clinical amebiasis if pharmacological and toxicological properties, which are yet to be determined, permit.

It was disappointing to find that some of the most highly active antimalarials such as metachloridine (2-metanilamido-5-chloropyrimidine (Table 4, XXXVII)), paludrine (1-p-chlorophenyl-5-isopropylbiguanide (Table 4, XLI)) and plasmochin (Table 4, XLVI) are essentially inactive amebastatically. This simply demonstrates further the specificity of their action.

Regardless of whether an amebastatic drug can be practical, testing for amebastasis rather than amebacidal activity markedly increases the sensitivity of the test since

the "static" endpoint for any drug is a much lower concentration than the "cidal" endpoint. This minimizes certain difficulties such as those encountered with drugs of low solubility and makes possible the testing of new and soluble compounds at many times the effective concentration of standard drugs. Also, this increased sensitivity justifies reading the endpoint arbitrarily as the presence or absence of growth rather than attempting the more subjective estimation of amebae density, with results expressed by the multiple plus system, or using the more time-consuming dilution counting procedures. The use of small numbers of amebae for inoculation likewise simplifies the testing program because one tube is sufficient to inoculate at least one hundred experimental tubes so it is not necessary to maintain such large numbers of stock culture tubes.

As is the case with every *in vitro* drug test, there is never complete assurance of good carry-over of results to *in vivo* conditions. This is illustrated in amebiasis by chiniofon, which, while effective clinically (Craig, 1944), is only mildly active in our *in vitro* tests (Table 4) and in *in vitro* tests of others (David et al, 1933).

A number of compounds was tested for both antimalarial and amebastatic activity and the activity in these two tests has been compared. Of the nineteen compounds which possessed some degree of antimalarial activity eleven also had amebastatic activity. Eleven other compounds had amebastatic but not antimalarial activity. The greatest lack of correlation was seen in metachloridine, paludrine, and plasmochin, all of which have high antimalarial activity but little or no amebastatic activity. About the only safe conclusion to be drawn is that compounds showing activity in other infections should be tested for amebastatic activity, but there is no assurance of positive results.

There are reports in the literature that emetine is amebacidal at very high dilutions such as 1 : 1,000,000 (0.1 mg/100 cc) (St. John, 1933) which is considerably different from our findings and those of Anderson and Chuan (1944). It is to be noted that the medium used by St. John was fluid and of relative simplicity. Anderson (1946) and his colleagues have compared the relative activities of a number of compounds against amebae grown in egg-slope medium and in a liquid-liver medium, and found that a number of drugs were more active in the latter medium. The amebacidal concentration of emetine in egg-slope medium was between 5 and 10 mg per 100 cc (1 : 20,000–1 : 10,000) while in the liquid medium emetine was amebacidal at about 0.5 mg per 100 cc (1 : 200,000). It is suggested by Anderson (personal communication) that the egg-slope medium adsorbs a large proportion of the drug, rendering it unavailable for action against the amebae. Hansen and Anderson, 1946, have described a liquid medium of essentially known ingredients. Either this or the liquid-liver medium may prove to be preferable for drug testing. It is felt that the conclusions drawn in this paper regarding the usefulness of small inocula in cultures of *E. histolytica* would apply if the amebae were grown in either of these liquid media, in which case an even more sensitive testing procedure would be the result.

Incorporating what we consider to be the best features from our studies as well as those of others, for the cultivation of *E. histolytica* and for testing drugs against it, the following key has been devised for routing a compound through the proper tests.

Attempts to evaluate test media in studies of the nutrition of *E. histolytica* are handicapped when large inocula are used because of the effect of material carried over from the stock cultures in the inoculum, and by the fact that in order to determine whether there is more than survival of the parasites it is necessary to make three to

Routine Screening

1. Inoculate with 500 ± amebae 4 tubes liquid amebae culture medium containing 16 mg test compound/100 cc — go to 2
2. 18 hours later: Record turbidity ("t" organism growth) — go to 3
3. 96 hours. Record turbidity
   Examine sediment of two tubes for amebae:
   a. Amebae present — go to 4
   b. Amebae absent — go to 5
4. Examine remaining two tubes immediately: if more than 2/4 positive. — Compound is inactive
5. 120 hours: Examine remaining two tubes:
   Record turbidity
   a. Amebae present — Compound is inactive
   b. Amebae absent — go to 6

Titration

6. Titrate drug using four-fold dilutions as follows: 16, 4, 1, and 1/4mg/100 cc
   Inoculate tubes with 500 ± amebae — go to 7
7. 18 hours later: Record turbidity — go to 8
8. 96 hours: Record turbidity
   Examine sediment for amebae:
   a. No amebae in any tubes — go to 9
   b. Amebae in some tubes — go to 10
9. Titrate further, using four-fold dilutions 1/4, 1/16, 1/64, 1/256 mg/100 cc — continue or go to 10
10. Triplicate sets of four dilutions, two-fold steps straddling the minimal inhibitory concentration (M.I.C.) found in 8 or 9. Read at 96 hours. — record MIC and go to 11
11. Subinoculate from all negative tubes in 10 and read for amebae growth at 72 and 120 hours. — go to 12

Amebacidal Test

12. Treat four 72-hour cultures (inoculum 2,000 amebae) with four times the M.I.C. If this is less than 32 mg% treat another four with 50 mg% — go to 13
13. 6 hours: Examine sediment of two tubes of each set:
    a. No amebae (examine other two tubes) — Amebacidal
    b. Amebae present — go to 14
14. 24 hours: Examine all four tubes
    a. No amebae — Amebacidal
    b. Amebae present — go to 15

Direct or Indirect Action

15. Special tests to determine whether drug is acting directly or indirectly.
    Earlier observations on "t" growth give some indication
    a. If "t" had flourished—probably *direct inhibitory* action
    b. If "t" growth questionable or absent—probably acting indirectly
    Run a bacteriostatic test using "t" organism and about four times the minimal inhibitory concentration of drug. Check for "t" inhibition by dilution count after sixteen hours' exposure to drug.

six transfers in the test medium. The use of small numbers (500 to 1000 per tube) of amebae for inoculations in these studies would reduce the amount of material carried over from the basic stock medium by one-hundred-fold or more, and since the amebae can only reach populations of appreciable size (200,000 to 500,000 per tube) by dividing many times, the original passage into the test medium is all that is necessary to determine whether the medium permits multiplication. With somewhat more effort the actual rate of multiplication can be determined for each test medium so that they can be graded as to their ability to furnish the requirements for the growth and multiplication of *E. histolytica*.

SUMMARY AND CONCLUSIONS

As few as 500 amebae of the species *E. histolytica* in association with the "t" organism invariably resulted in positive cultures in egg slope + Locke's overlay + rice starch medium, but as few as 15 amebae frequently gave positive cultures. The terminal population of amebae was independent of the number of amebae in the inoculum. The maximum fold increase in amebae populations seen in a single transfer was 36,000 or about fifteen generations. The average number of divisions per day under the conditions used was from two to two and one-half. Rapidly multiplying cultures were used to determine the amebastatic activity of standard and test compounds. Vioform and emetine were inhibitory at concentrations of about 0.5 mg/100 cc (1:200,000). Other compounds which were inhibitory at about these same concentrations are: para-aminobenzoic acid, 2-n-heptyl-4,6-diamino-s-triazine and 2-methyl-4-amino-5-bromomethylpyrimidine. These compounds act directly on the ameba since they were not inhibitory to the "t" organism. Sulfadiazine and related compounds are inhibitory but only due to an indirect action on the bacterial associate. Metachloridine (2-metanilamido-5-chloropyrimidine) is only active at high concentrations (100 mg/100 cc).

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FAILURE OF *AEDES AEGYPTI* AND *CULEX PIPIENS* TO TRANSMIT *PLASMODIUM VAUGHANI*

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Despite the intensive work which has been done on the avian plasmodia, many of the fundamental facts about these interesting parasites remain unknown. This is especially true of some of the smaller species which, for one reason or another, have not seemed promising research material to many investigators. Information about the mosquito transmission of numerous species of avian malaria is fragmentary, or completely lacking. *Plasmodium vaughani* is one of these species. Although known since its discovery by Novy and MacNeal in 1904, and perhaps the commonest internal parasite of robins, it has received very little study and no one has reported work on the species of mosquitoes responsible for its transmission.

To secure information on this problem, experiments were undertaken in the author's laboratory before the war and later were continued at the Army Medical School, in Washington, D. C. The first work was done by Ellen Counts (now Dr. Ellen C. Binckley), who fed 36 *Culex pipiens* on *P. vaughani*-infected canaries, and after intervals of from three to four weeks dissected the mosquitoes for both oöcysts and sporozoites. As a further check, the emulsified salivary glands were injected into clean canary birds. Thirty-two of the original 36 mosquitoes survived for microscopic examination, but the results of both dissection and inoculation were completely negative.[1] It is of interest, however, that this same strain of *Culex pipiens* proved susceptible to infection with both *Plasmodium relictum* var. *matutinum* and *P. rouxi*. The sporozoites of the latter species (which, as far as published reports go, have apparently not previously been seen) were found to be from 9 to 10 micra long, and from 0.5 to 0.8 micra in width, and were otherwise similar to the sporozoites of other species of malaria. That the sporozoites of *Plasmodium rouxi* should be so nearly like those of other species is rather remarkable, in view of the rather striking difference in size and morphology which this species exhibits when compared to others.

It may also be noted, in passing, that a number of these mosquitoes were allowed to bite birds infected with *Plasmodium nucleophilum*, *P. hexamerium*, and *P. oti* and that a few survived for as long as a month afterward, and were then allowed to feed on clean canaries and dissected. The salivary glands were also emulsified in saline and injected into healthy canaries. But all results were completely negative. However, since the number of surviving mosquitoes amounted to only 5 in each case, these results cannot be considered significant.

These experiments might have been continued but for the war. However, an opportunity for further work came during the fall of 1944 and the winter of 1945, while the author was stationed at the Army Medical School, Washington, D. C. During this period, two robins were caught in Rock Creek Park, which is near the School, and both were found heavily infected with *Plasmodium vaughani*. Gameto-

Received for publication, March 4, 1947.

[1] The strain of *P. vaughani* used originated in a robin caught near Syracuse in 1935. The strain of *Culex pipiens* was derived from larvae caught in a local rain barrel.

cytes of this species were very numerous in the blood. Unfortunately *Plasmodium relictum* var. *matutinum* was also present, but in each case few forms were seen and only occasional gametocytes.

The first of these robins was placed in a large screened cage containing *Culex pipiens* mosquitoes, which were then allowed to bite for several hours. It is impossible to say how many engorged females were observed afterward, but there were certainly many hundred, and the robin failed to survive the ordeal.[2]

During the following month a considerable number of the mosquitoes died, although they were kept in an air-conditioned insectary, but many survived, and these were then allowed to feed repeatedly on a total of 15 clean canary birds. It is not known how many did so, but many engorged females were seen afterward on each occasion. Finally those females which were still alive were dissected and the salivary glands examined for sporozoites. Nineteen of the 184 which were thus examined contained sporozoites, though in most instances the infections were light. Salivary glands from four of the more heavily infected mosquitoes were emulsified with saline, and injected into an equal number of clean canaries. However, no infections resulted, although 8 of the 15 birds which were directly exposed to the mosquitoes developed infections of *Plasmodium relictum* var. *matutinum*. Observations were continued for about six weeks, but in no case was there any evidence of infection with *Plasmodium vaughani*.

The second robin was exposed, in similar fashion to *Aedes aegypti* mosquitoes.[3] Although it was bitten by a large number (certainly many hundred), only a rather small fraction of this number survived at the end of the month following, chiefly because of the vagaries of a newly installed air-conditioning unit. Three fresh canaries were repeatedly exposed to these survivors, but no infections with any type of malaria resulted. The pressure of other work at that time prevented making any dissections. Since it is known that the number of individuals biting the exposed birds was considerable, however, it is likely that infections would have resulted if any large fraction of the mosquitoes had been susceptible to either of the two species of malaria concerned.

In considering these results, it is necessary to remember that mosquito susceptibility to malarial infection is influenced both by the strain of mosquito used and probably by the strain of parasite also. And it is also true that *Plasmodium vaughani* is one of the few species of avian plasmodia which exhibit a rather high degree of host specificity, being apparently almost wholly restricted in nature to the robin (although it does occur, at least occasionally, in other species of hosts, as for example, the brown thrasher). The fact that the canary is susceptible to blood-induced infections does not prove that it would become infected as the result of the bites of susceptible mosquitoes. It seems likely that some host species are hospitable to the erythrocytic stages of some types of malaria, but do not prove fertile ground for the development of the stages arising directly from the sporozoite. Such a case is that of the White Pekin duck, which is intensely susceptible to *Plasmodium circumflexum*,

[2] It is worth noting that the brain of this robin showed numerous exoerythrocytic forms. Since its blood contained many more *vaughani* forms than those of *relictum*, it is believed likely that these exoerythrocytic stages were of the former species. If this is the correct interpretation, this is the first instance in which such stages of *P. vaughani* have been seen.

[3] The *Culex pipiens* strain was one of local origin, having been isolated from larvae caught in the neighborhood of Silver Spring, Md. The *Aedes aegypti* strain came from the National Institute of Health.

when this species is introduced in blood, but has never been reported naturally infected.

It is therefore not certain that these negative results prove that the species of mosquitoes used are never vectors in nature. It does seem likely, however, that neither *Culex pipiens* nor *Aedes aegypti* is sufficiently susceptible to infection with *Plasmodium vaughani* to be a transmitting agent of any importance. For conclusive results, more mosquitoes should be employed, preferably of different strains, and a species of bird known to be susceptible to sporozoite infection should also be employed. This would mean some species known to be infected in nature, though the use of robins would certainly be difficult, since they do not stand captivity at all well.

RESEARCH NOTES

NEW RECORDS OF HELMINTHS IN CHICKENS FROM TENNESSEE

In the course of a survey of the helminths of chickens in Tennessee two species of parasites have been recovered which perhaps constitute new records of their occurrence in chickens in the United States.

Echinoparyphium recurvatum (von Linstow), a trematode parasite of the small intestine of domesticated and wild ducks, fowls, and pigeons, has been reported in this country from turkeys in California by Annereaux (1940, J. Am. Vet. Med. Assoc. **96**(754) : 62–64). Two specimens of this species were recovered from a Barred Plymouth Rock hen examined in Washington County, Tennessee.

The tapeworm, *Fimbriaria fasciolaris* (Pallas, 1781) is reported by Mönnig (Veterinary Helminthology and Entomology, Wm. Wood & Co., New York, 1934) to occur in the small intestine of the fowl, duck, goose, and many wild birds. The worm is notable because it possesses a pseudo-scolex which develops from an anterior expansion of the body and replaces the true

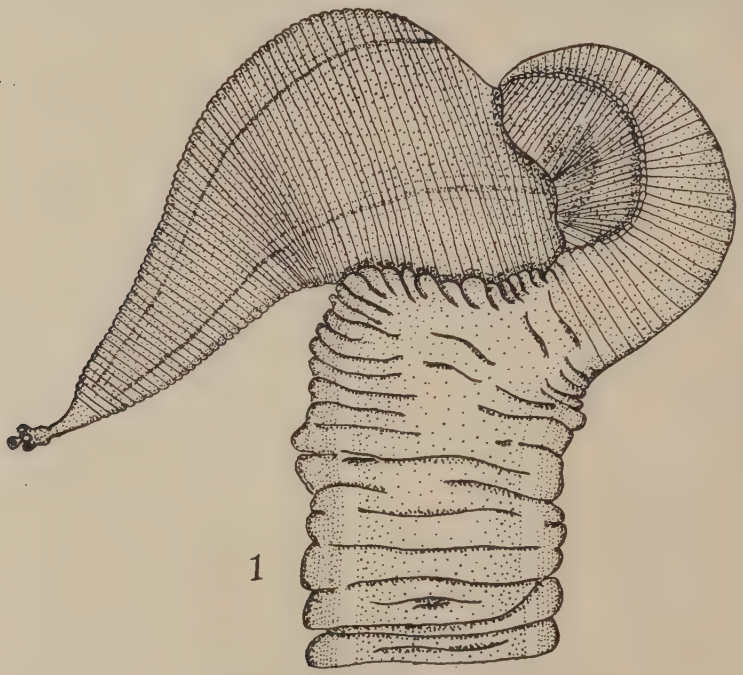

FIG. 1. Scolex and pseudo-scolex of *F. fasciolaris*.

scolex in function (Fig. 1). Eight specimens were recovered from a Rhode Island Red chicken examined in Davidson County, Tennessee. These ranged in length from 14 to 85 mm, and only one had retained the true scolex.—A. C. TODD, *Tennessee Agricultural Experiment Station, Knoxville.*

HOOKWORM INFESTATIONS IN AUSTRALIAN SOLDIERS*

To the Editorial Committee of the JOURNAL OF PARASITOLOGY:

In a recent article dealing with the distribution of human hookworm species in the Pacific Islands, Stoll (1946, J. Parasitol. **32**: 490) has questioned the accuracy of results published by Lowe and Lancaster (1944, Med. J. Australia **1**: 289) from soldiers who had served in islands north of Australia. He has wrongly assumed that the three species of hookworms referred to in

* Received for publication, February 14, 1947.

this paper—*Necator americanus*, *Ancylostoma duodenale*, and *A. braziliense*—were diagnosed on the basis of egg measurements alone.

The actual work of identifying the genera, and, in some of the specimens, separating the species *duodenale* and *braziliense*, was done at this School with fecal specimens sent by Dr. Lancaster; our results were forwarded to him.

I agree with Dr. Stoll that egg measurements do not give a practical method for separating the species but there are other easy ways of reaching the same end. At this School for many years we have examined the third-stage larvae (obtained by culturing fecal specimens containing eggs) to separate *Necator* from *Ancylostoma* infections. The appearance of the buccal structure of a larva determines the genus (*Necator* or *Ancylostoma*) with certainty, except in the rare cases in which it is obscured. Another character which enables a very probable but not quite certain diagnosis of the genus is the relative position of the genital rudiment. These and other criteria for the separation of third stage larvae of *Necator* and *Ancylostoma* have been discussed by various authors (Thiel, 1923, Tijd. Vergel. Geneesk. **10**: 285; Svennson and Kessel, 1927, J. Parasitol. **13**: 146; Heydon, 1927, Med. J. Australia **1**: 531; Eisma, 1932, Thesis, abstracted Trop. Dis. Bull. 1932, **29**: 593).

In some of the above cases *Ancylostoma* larvae were fed to kittens and two of them began passing hookworm eggs within four weeks. Both were found post mortem to be *A. braziliense* infections; one had 8 males and 9 females, the other 7 males and 12 females. *A. braziliense* is common in cats and dogs in North Queensland but we have never found natural infections in these animals near Sydney. It is certain that the kittens used in these feeding experiments could have been infected in no other way.—A. J. Bearup, *School of Public Health and Tropical Medicine, Sydney, Australia.*

CONCERNING REPORTS OF *ANCYLOSTOMA BRAZILIENSE*

To the Editorial Committee of the Journal of Parasitology:

The reservation (Stoll, 1946, J. Parasitol. **32**: 490) as to the validity of the hookworm species recorded for Australian soldiers by Lowe and Lancaster (1944, Med. J. Australia **1**: 289) can be withdrawn, in the light of Bearup's communication above specifying the use of larval criteria for generic differentiation, and the use of the cat as a host to test for the presence of *Ancylostoma braziliense* in some of the cases. I was, perhaps, over-impressed by a certain casualness in Lowe and Lancaster's brief paragraph and table referring to them, where the genus was thrice spelled "*Ankylostoma*," and the 41 hookworm infections were allocated not quite clearly to the 29 cases.

It seems fair to require a statement of the type of evidence used for adding to the record additional infections with adult *A. braziliense* in man. Though Oiso (1930, Taiwan Igak. Z. **22**: 28) successfully parasitized 7 human volunteers with infective larvae, using both mouth and skin routes, and later recovered as adult worms after treatment 5 to 74 per cent of the number of larvae administered, Dove (1928, J. Parasitol. **15**: 136) failed in a test with a human volunteer, using larvae cultured from the feces of a Texas boy with a verified *A. braziliense* infection. Even with the new interest aroused in *A. braziliense* by reason of its relation to creeping eruption, there appear to be less than 200 known instances of the natural occurrence of the adult parasites in man. In addition to those summarized by Darling (1924, Am. J. Hyg. **4**: 416), there is the Texas case referred to above, the Burma cases noted by Chandler (1929, Hookworm Disease), and a verification by Bonne (1937, Am. J. Trop. Med. **17**: 587) of its presence in a Javanese (although Helm. Abs., 1942, **11**: 49, incorrectly ascribes 5 additional cases to Bonne in Java).

Altogether only about 400 adult *A. braziliense* have been identified from man, in somewhat more than 80,000 adult hookworms examined, this total including only those lots of worms reported as containing some *A. braziliense*. In the western hemisphere, the adult worms in man have been confirmed only from Brazil and Texas. In the eastern hemisphere the records include Bengal, Burma, Siam, Malay Peninsula, Sumatra, Java, Philippine Islands, Formosa (?), Fiji, and, currently, "islands north of Australia." The relatively restricted areas in which *A. braziliense* has been verified in its adult form in man stand in contrast to its more extensive distribution in cat, dog, and related hosts. This discrepancy is suggestive of a similar discrepancy in the infrequent reports of *Trichinella spiralis* in man in the Orient as compared to its incidence in reservoir hosts.—Norman R. Stoll, *The Rockefeller Institute for Medical Research, Princeton, N. J.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Sixth Council Meeting, Boston, Massachusetts

December 26, 1946

The meeting of the Council of the American Society of Parasitologists was called to order by President N. R. Stoll at 7:00 PM, December 26, 1946, in the Faculty Club of Harvard University, Cambridge, Massachusetts. Past-Presidents J. E. Ackert, G. R. LaRue, and D. H. Wenrich, and the following members of Council were present: T. W. M. Cameron, G. R. Coatney, J. T. Culbertson, H. Kirby, Jr., H. W. Manter, G. F. Otto, E. W. Price, R. M. Stabler, N. R. Stoll, H. W. Stunkard, and W. H. Wright. In addition, E. C. Dougherty and P. D. Harwood were present at the invitation of Council for the presentation of special reports.

The regular order of business was set aside in order that the special reports of E. C. Dougherty and P. D. Harwood could, first, be heard. Dr. Dougherty described the inquiries he had made in the National Institute of Health and the Bureau of Animal Industry as to why the Index-Catalog of Medical and Veterinary Zoology had not been published in full and asked whether this Society would be willing to go on record as favoring the early publication of the catalog. One Councillor stated that the government agencies fully appreciated the need for completion of the Index-Catalog publication, but that the limited present budget of the principal agency concerned could not pay for such publication. He urged that an appeal be made by those interested in the Index-Catalog to different foundations which might supply the necessary funds for prompt publication. It was finally moved and seconded that Council endorse the completion of the author catalog and the early publication of the subject catalog; and that letters describing this action be sent to the Secretary of Agriculture, the Director of the Budget, and the Chairman of the Subcommittee on Agriculture of the Appropriations Committee of the House of Representatives. The motion passed.

Dr. Harwood reported as Chairman of the Committee on Common Names. He described the need for "official" common names, especially for the helminths, and referred to a list of such names which his committee (which included D. B. McMullen and the late H. L. Van Volkenberg) had prepared. Various comments were made by those present praising the committee for its effort, but generally questioning the propriety of the Society recommending common names for parasites. Deficiencies and inaccuracies of the list which had been prepared were pointed out. It was suggested that J. R. Christie be added to the committee so as to check on names of the plant helminths. Finally, G. F. Otto moved that the report of the committee be accepted subject to improvement of specified deficiencies and inaccuracies. The motion passed after J. E. Ackert had urged that, if the report were printed, the lists of scientific names, which had been arranged for the three principal classes of helminths, precede the list of common names.

The regular order of business was then taken up.

I. Reports of Officers

1. *Secretary (J. T. Culbertson):* As of December 20, 1946, there were 512 active members of the Society in good standing, of whom 463 lived within and 49 lived outside continental United States. Fifty persons were elected to membership during the year, of whom 46 lived within and 4 lived outside continental United States. Since organization of the Society, 1335 persons had been elected to active membership. The active membership as of December 20 represented 38 per cent of the total number of electees. One member, Dr. C. J. Addis, of Little Rock, Arkansas, died during the year. The report was accepted and placed on file.

2. *Treasurer (R. M. Stabler):* The Treasurer's report for the fiscal year (March 2, 1946, to December 1, 1946) follows:

Receipts

Balance on hand March 1, 1946		$7865.88
Collected during current year:		
92 members dues applying 1946	$ 369.25	
9 members dues applying 1947	36.50	
3 members dues applying after 1947	9.00	
10 dues in arrears	76.25	
37 applicants	153.00	
		$ 643.00

228 subscriptions to Vol. 32	1034.51		
172 subscriptions to Vol. 33 and after	791.25		
Sales of individual numbers (Vol. 32)	17.70		
		1843.46	
Back volumes and numbers	811.47		
Advertisements	83.40		
Author's charges	282.39		
Index, volumes 1–25	45.75		
Interest on savings account	8.60		
Miscellaneous	36.00		
		1267.61	
			3754.07
			$11619.95

Debits

Printing and handling journal			
Volume 31	$1670.69		
Volume 32	3762.08		
		$5432.77	
Expenses in office, Chairman of Editorial Committee	87.70		
Expenses in office of Secretary	106.56		
Expenses in office of Treasurer	618.28		
Expenses in office of Custodian of Back Numbers	164.27		
Expenses in office of President	4.80		
		981.61	
Printing Back Numbers (Vol. 30, No. 3)	187.68		
Printing portraits	10.98		
		198.66	$ 6613.04
Balance on hand, December 1, 1946			5006.91
			11619.95

Audited and Found Correct, December 26, 1946,
by Auditing Committee:
(signed) T. W. M. CAMERON
(signed) E. W. PRICE.

By way of analysis and comparison with the previous report, the Treasurer made the following statements:

"1. Last year's report covered 15 months, and recorded one of the biggest financial years for the Society, with the total funds for the year being $13,272.74.
"2. At that, the total funds for the current fiscal year are not far behind, at $11,619.95.
"3. This year's books actually show a deficit of $2858.97.
"4. Some factors contributing to this deficit are:
   a. Of 502 member's dues applying in 1946, 410 had already been paid by March 1, 1946.
   b. Of 673 subscriptions applying in 1946, 445 had paid by March 1, 1946.
   c. It cost the Society something short of two thousand dollars ($1806.29) more to print the Journal in 1946 ($5432.77) than in 1945 ($3626.48).
"5. Despite the superficially depressing effect of the deficit, the cash balance for 1946 ($5006.91) is not alarmingly below 1945 ($7865.88)."

Following motion, the report of the Treasurer was accepted, subject to audit, and placed on file.

II. REPORTS OF CUSTODIANS AND COMMITTEES

1. *Custodian of the Princeton Secretarial Fund (N. R. Stoll):* The report covering the period March 11, 1946, to December 1, 1946, follows:

Savings bank balance, March 11, 1946	$1052.88
Interest credited	5.26
Savings bank balance, December 1, 1946	$1058.14

Audited and found correct, December 26, 1946,
by Auditing Committee:
(signed) E. W. PRICE
(signed) T. W. M. CAMERON.

The report was accepted subject to audit.

2. *Chairman of the Editorial Committee (H. W. Stunkard):* The present report includes a financial statement for only the first four issues of Volume 32, since bills for later issues have not yet been received. The cost of the four issues already paid for was as follows:

February issue, 1400 copies	$ 741.47
April issue, 1400 copies	915.28
June issue, 1410 copies	998.43
August issue, 1400 copies	1173.27

There was no change from previous years in editorial policy. The current volume will run over 600 pages compared with 456 pages in Volume 31, not including the Supplements. In order to conform to postal regulations, the format of the cover of the Supplement has been changed. The Supplement is now considered "Section 2" of the December issue. Difficulties in maintaining printing schedules were emphasized, although improvement was reported in 1946 compared with 1945.

Charges at the Press were increased 10% on July 1. It is anticipated that in the future printing costs will be substantially larger than formerly, and that a larger number of suitable manuscripts will be received for publication. It is estimated that 600 pages of Journal may cost between $6000 and $7000 annually. The grant of $1000 from the American Foundation for Tropical Medicine has not yet been used.

Advertising rates are still low compared with other journals, and it is advised that these be increased.

The subject of reviews of books has continued to be perplexing to the Editorial Committee and advice was requested on the printing of such reviews.

Authors have been assessed one-half the cost of publication for that part of papers exceeding ten pages.

G. F. Otto moved acceptance of the report, with thanks to the Editorial Committee and especially to its Chairman. Motion carried.

In the discussion which followed, the consensus opposed printing book reviews. Discretion was left with the Committee as to advertising rates, authors' charges, and overall editorial policy.

3. *Custodian of Back Numbers (G. F. Otto):* Five issues of the Journal, for which stock was depleted, were reprinted during 1946. One hundred sixty-eight complete volumes of the Journal were received as gifts, as were 85 separate issues. It was recommended that 2 additional issues, now low in stock, be reprinted. The report was accepted.

4. *Auditing Committee (T. W. M. Cameron and E. W. Price):* The reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund were certified to be correct.

5. *Special Standing Committees:* The committees on nomenclature and avian malaria had nothing to report. The report of the committee on common names was considered earlier.

III. Reports of Representatives

1. *To Council of A.A.A.S. (W. H. Wright and G. R. Coatney):* No formal report was made, but it was announced that the A.A.A.S. would hold its next annual meeting in Chicago, Illinois, during December, 1947.

2. *To Council of U.A.B.S. (D. L. Augustine and R. M. Stabler):* No report was submitted.

3. *To Committee on International Congress on Tropical Medicine (Communication from W. W. Cort):* During May, 1946, the representatives of the various societies interested in the promotion of an international congress on tropical medicine met in Washington, D. C., with Mr. Warren Kelchner of the Department of State, who urged that steps be taken to make the proposed congress officially the "Fourth International Congress on Tropical Medicine." Such authorization was later obtained by Dr. Mark Boyd. The United States Government through the State Department was then requested officially to sponsor this Congress. At the end of October, the United States Government decided to accept such sponsorship, and a meeting of representatives of the interested societies was called for January 3, 1947, to consider organization of the Congress. The report was accepted and placed on file.

4. *To National Research Council (E. W. Price):* The annual meeting of the Division of Biology and Agriculture of the National Research Council was held April 13, 1946. The proposed Institute of American Biologists was discussed at length, need for such an organization being generally accepted. Four classes of membership in the Institute were proposed:

Individual members—professional biologists;
Student associates—undergraduate and graduate students;
Contributing members—chemical firms, drug manufacturers, etc.;
Member Societies—societies of professional biologists.

The services of the proposed Institute were considered legitimately to be: (a) publication of a journal, known as the American Biologist; (b) operation of a placement office; (c) arrangement of meetings for societies; (d) solicitation of funds for publication of worthy biological materials; (e) represent biologists whenever such representation is required.

It was related that the Science Foundation bill (S 1850) passage of which this Society had supported had been approved by the Senate of the Seventy-Ninth Congress, but had not gained approval in the House of Representatives.

The report was accepted and placed on file.

IV. New Business

1. *Election of New Members:* The following persons were elected to membership in the Society: Wilton Monroe Fisher, Baylor University; Sister Francis Soltano Geisler, Nazareth College; J. Steger Hunt, University of Michigan; Glenn Lyle Hoffman, University of Iowa; Cluff E. Hopla, Brigham Young University; Everett Williams Jameson, Jr., Cornell University; Irving George Kagan, University of Michigan; Don Wilfred Micks, Johns Hopkins University; Milton Gottlieb Mueller, University of Michigan; Gordon E. Nielson, University of Iowa; Robert B. Short, University of Michigan; Richard Dean Stone, University of Iowa; and Anne Van der Woude, University of Michigan.

2. *Extension of Tenure of representatives to A.A.A.S.:* Council voted that representatives of the Society to the Council of A.A.A.S. should hereafter be appointed for two years.

3. *Requests for Donations:* It was voted to contribute ten dollars to the American Society of Naturalists to support the Biologists' Smoker at the Boston meeting.

4. *Plans for Next Meeting:* Council voted to meet with A.A.A.S. in Chicago, in 1947.

5. *Nominations, Elections, and Appointments to Society Offices:*

   a. The following persons were nominated by Council for the designated offices in the Society: President for 1947, H. J. Van Cleave; Vice-President for 1947, C. G. Huff; Treasurer for 1947 and 1948, R. M. Stabler; Councillors-at-Large for four years, E. W. Price and M. D. Young. C. B. Philip was nominated to succeed C. G. Huff as Councilor-at-Large, in the event of Dr. Huff's election as Vice-President.

   b. The following persons were elected by Council to the Editorial Board of the Journal of Parasitology: R. J. Porter, W. Trager, and A. C. Walton.

   c. President N. R. Stoll appointed, for 1 more year, W. H. Wright and G. R. Coatney as representatives of the Society to the Council of A.A.A.S.

      Following Council approval, Dr. Stoll appointed for one year: Dr. W. Malcolm Reid as representative of the Society to serve on the Committee on Materials for Visual Instruction in Microbiology, of the Society of American Bacteriologists (Dr. H. E. Morton, Chairman, Department Bacteriology, University of Pennsylvania School of Medicine).

      Dr. Stoll also appointed as a Special Committee on Nominations for Secretary and Chairman, Editorial Committee J. P., to report at next annual meeting: A. C. Chandler, G. R. Coatney, C. G. Huff, H. Kirby, E. W. Price, R. M. Stabler, W. Trager and G. F. Otto, chairman.

   d. No changes were made in the membership of the Special Standing Committees.

At 11:00 PM, Council voted to adjourn.

Respectfully submitted,
James T. Culbertson, *Secretary.*

Twenty-First Annual General Business Meeting

December 27, 1946

The general business meeting of the Society was called to order by President N. R. Stoll at 1:30 PM, immediately following the annual luncheon at the Longwood Towers, Brookline, Massachusetts. One hundred and four persons were present. The reports of the Secretary, Treasurer, Custodian of the Princeton Secretarial Fund, Chairman of the Editorial Committee, and Custodian of Back Numbers were read and accepted. Dr. E. C. Faust described the status of the proposed International Congress on Tropical Medicine. The representative to the National Research Council described the present status of plans for the formulation of the American Institute of Biology. Dr. E. C. Dougherty outlined his interest in advancing the publication of the Index-Catalog of Medical and Veterinary Zoology, and President Stoll stated the action taken by Council in behalf of the Index-Catalog. Dr. Harwood appealed for suggestions with respect to the work of his committee on common names.

The Society was asked to approve an amendment to the Society Constitution. Council had previously voted to change the requirement for active membership in the second article of the Constitution to read:

> "Any person with suitable educational qualification interested in parasitology may be a candidate for active membership."

It was moved and seconded that the proposed constitutional amendment be adopted by the Society. Approval was unanimous.

The Secretary read the names of the persons nominated by Council to the Society offices. There being no nominations from the floor, it was moved and duly seconded that the Secretary cast one ballot for the slate of nominees. The motion carried. Accordingly, the officers for 1947 are: President, Harley J. Van Cleave; Vice-President, Clay G. Huff; and Treasurer (to serve through 1948), Robert M. Stabler. E. W. Price and M. D. Young were elected to serve on the Council for four years, and C. B. Philip was elected to serve on Council, in place of C. G. Huff, for 1947.

The Secretary then read the names of the three new members of the Editorial Board of the Journal.

A vote of thanks was extended to Dr. D. L. Augustine for his services as local representative of the Society in arranging for the Boston meeting.

The Society voted to adjourn at 2:30 PM, to meet again at the A.A.A.S. convention in Chicago, in December, 1947.

Respectfully submitted,
JAMES T. CULBERTSON, *Secretary*.